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WO 91/06667 [redacted]
WO91/06666

09/203500
A#21

1. Document ID: US 6162638 A

L12: Entry 1 of 45

File: USPT

Dec 19, 2000

US-PAT-NO: 6162638
DOCUMENT-IDENTIFIER: US 6162638 A
TITLE: Attenuated strains of leishmania and uses thereof
DATE-ISSUED: December 19, 2000

US-CL-CURRENT: 435/258.3; 424/184.1, 424/265.1, 424/269.1, 424/93.1, 424/93.2, 424/93.21, 435/258.1, 435/320.1, 536/23.2

APPL-NO: 8/ 643751
DATE FILED: May 6, 1996

IN: Papadopoulou; Barbara, Ouellette; Marc, Olivier; Martin

AB: Attenuated strains of Leishmania are provided in which at least one gene contributing to virulence of the strain and expressed in both the promastigote and amastigote forms of the strain is functionally disabled, such as, by deleting at least a portion of the gene or by mutagenesis of the gene. The attenuated strain may be used for administration to a host to confer protection against disease caused by a virulent Leishmania strain or as a diagnostic reagent.

L12: Entry 1 of 45

File: USPT

Dec 19, 2000

DOCUMENT-IDENTIFIER: US 6162638 A
TITLE: Attenuated strains of leishmania and uses thereof

DEPR: To look at how this novel fragment has arisen, chromosomes of the TR/hyg/neo transfectant were resolved by CHEF electrophoresis and the blot was hybridized with the appropriate probes. In FIG. 1(C), there is shown a molecular karyotype of diploid and triploid L. donovani TR transfectants. Chromosomes were separated by pulsed-field gel electrophoresis using a Biorad CHEF-DR III apparatus at 5 V/cm, 120.degree. separation angle and switch times varying from 35-120 for 27 h. Lanes 1: L. donovani WT strain; 2: L. donovani TR/hyg single knockout strain; and 3: L. donovani TR/hyg/neo double targeted mutant. Blots were hybridized with TR, hyg and neo-specific probes as shown. In addition to a 520 kb chromosome on which the TR gene is normally located, a new chromosome of approximately 1200 kb hybridized to a TR specific probe (FIG. 1(C), lane 3). The third allele present in the new chromosomal location correspond to the one targeted by the neo gene (see FIG. 1(C), lane 3), thus leaving an intact TR allele in the initial chromosomal location (520 kb). Therefore this attempt to generate a TR null mutant in L. donovani has failed and, in an additional independent transfection, we were also unable to generate a null mutant as the locus became triploid. This may suggest that the TR gene is an essential gene. To test whether the polyploidy of the TR locus occurred only in a pathogenic virulent strain as previously described for the dhfr-ts gene of one virulent L. major strain (ref. 11), we have attempted to disrupt the TR gene of the non-pathogenic lizard strain L. tarentolae. Thus, referring to FIG. 2, there is schematically illustrated the inactivation of the TR gene in Leishmania tarentolae. Constructs derived from one species are not appropriate for the targeting of genes of another species of Leishmania. Therefore, the TR gene of L. tarentolae was amplified by PCR and the hyg and neo expression cassettes were cloned into the BalI site of the PCR fragment and transfections were performed to inactivate both alleles of the L. tarentolae TR gene. Referring to FIG. 2A there is a schematic drawing of the TR locus in L. tarentolae. Following integration of the hyg gene at the homologous locus, two additional EcoRI (RI) fragments of 8.75 and 3.8 kb would be generated and after the neo insertion two EcoRI fragments of 8.5 and 3.8 kb.

2. Document ID: US 6130364 A

L12: Entry 2 of 45

File: USPT

Oct 10, 2000

US-PAT-NO: 6130364
DOCUMENT-IDENTIFIER: US 6130364 A
TITLE: Production of antibodies using Cre-mediated site-specific recombination
DATE-ISSUED: October 10, 2000

US-CL-CURRENT: 800/6; 435/326, 435/463, 435/69.1, 800/13, 800/18, 800/4

APPL-NO: 8/ 412826
DATE FILED: March 29, 1995

IN: Jakobovits; Aya, Zsebo; Krisztina M.

AB: A method to produce a cell expressing an antibody from a genomic sequence of the cell comprising a modified immunoglobulin locus using Cre-mediated site-specific recombination is disclosed. The method involves first transfecting an antibody-producing cell with a homology-targeting vector comprising a lox site and a targeting sequence homologous to a first DNA sequence adjacent to the region of the immunoglobulin loci of the genomic sequence which is to be converted to a modified region, so the first lox site is inserted into the genomic sequence via site-specific homologous recombination. Then the cell is transfected with a lox-targeting vector comprising a second lox site suitable for Cre-mediated recombination with the integrated lox site and a modifying sequence to convert the region of the immunoglobulin loci to the modified region. This conversion is performed by interacting the lox sites with Cre in vivo, so that the modifying sequence inserts into the genomic sequence via Cre-mediated site-specific recombination of the lox sites. Also disclosed are a form of the method used to produce a cell expressing a modified antibody molecule using Cre-mediated site-specific recombination, and antibody-producing cells obtainable by the disclosed methods. Class-switching modifications of human antibodies produced in murine hybridoma cells are exemplified.

L12: Entry 2 of 45

File: USPT

Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130364 A
TITLE: Production of antibodies using Cre-mediated site-specific recombination

DEPR:
The invention method in Scheme A requires integration of only a single lox site adjacent to the
constant region gene to be modified. In this scheme, a first lox site is integrated adjacent to
the constant region gene (human C.sub..mu.) which is to be converted to a modified constant
region (human C.sub..gamma.), via site-specific homologous recombination with genomic DNA. A
vector (comprised of a circular DNA) comprising a second lox site and a selectable marker gene
(DHFR) is then transfected into the cells, and the lox sites are transiently interacted with Cre.
Cre is provided by cotransfection of a vector which transiently expresses a cre gene, as
described above. Cre-mediated site-specific recombination of the lox site in the genome with the
lox site on the circular vector results in insertion into the genome of the modifying sequence
(C.sub..gamma.) with the selectable marker gene (DHFR). The desired transfectants, which have two
lox sites flanking the DHFR and C.sub..gamma. genes, are stable in the absence of further Cre
expression and are obtained by selecting for expression of the DHFR marker gene (i.e. resistance
to the drug methotrexate indicated as "Met.sup.R " in FIG. 1). Verification of the desired
modifications of the immunoglobulin loci after each transfection and selection step is provided
by conventional genetic analyses, such as by PCR amplification, as described above.

3. Document ID: US 6121022 A

L12: Entry 3 of 45

File: USPT

Sep 19, 2000

US-PAT-NO: 6121022
DOCUMENT-IDENTIFIER: US 6121022 A
TITLE: Altered polypeptides with increased half-life
DATE-ISSUED: September 19, 2000

US-CL-CURRENT: 435/69.7; 435/320.1, 435/328, 435/334, 435/69.6, 530/350, 530/387.3, 530/388.22,
536/23.4, 536/23.53

APPL-NO: 8/ 422112
DATE FILED: April 14, 1995

IN: Presta; Leonard G., Snedecor; Bradley R.

AB: Polypeptides that are cleared from the kidney and do not contain in their
original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding
epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

L12: Entry 3 of 45

File: USPT

Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6121022 A
TITLE: Altered polypeptides with increased half-life

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

4. Document ID: US 6121415 A

L12: Entry 4 of 45

File: USPT

Sep 19, 2000

US-PAT-NO: 6121415
DOCUMENT-IDENTIFIER: US 6121415 A
TITLE: ErbB4 receptor-specific neuregolin related ligands and uses therefor
DATE-ISSUED: September 19, 2000

US-CL-CURRENT: 530/324; 530/350, 530/399

APPL-NO: 8/ 899437
DATE FILED: July 24, 1997

PARENT-CASE:
RELATED APPLICATION This application is a non-provisional application filed under 37 CFR
1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60/052,019 filed
Jul. 9, 1997, the contents of which are incorporated herein by reference.

IN: Godowski; Paul J., Mark; Melanie Rose, Zhang; Dong Xiao

AB: The invention concerns a novel neuregulin related ligand (NRG3) including
fragments and variants thereof, as new members of the neuregulin family of compounds. The
invention also concerns methods and means for producing NRG3. The native polypeptides of the
invention are characterized by containing an extracellular domain including an EGF-like
domain, a transmembrane domain and a cytoplasmic domain. Isolated nucleotide sequences
encoding such polypeptides, expression vectors containing the nucleotide

sequences,
recombinant host cells transformed with the vectors, and methods for the recombinant
production for the novel NRG3s are also within the scope of the invention.

L12: Entry 4 of 45
File: USPT
Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6121415 A
TITLE: ErbB4 receptor-specific neuregolin related ligands and uses therefor

DRPR:
It is further envisioned that the NRG3 of this invention may be produced by homologous
recombination, as provided for in WO 91/06667, published May 16, 1991. Briefly, this method
involves transforming a cell containing an endogenous NRG3 gene with a homologous DNA, which
homologous DNA comprises (a) an amplifiable gene (e.g. a gene encoding dihydrofolate reductase
(DHFR)), and (b) at least one flanking sequence, having a length of at least about 150 base
pairs, which is homologous with a nucleotide sequence in the cell genome that is within or in
proximity to the gene encoding NRG3. The transformation is carried out under conditions such that
the homologous DNA integrates into the cell genome by recombination. Cells having integrated the
homologous DNA are then subjected to conditions which select for amplification of the amplifiable
gene, whereby the NRG3 gene is amplified concomitantly. The resulting cells are then screened for
production of desired amounts of NRG3. Flanking sequences that are in proximity to a gene
encoding NRG3 are readily identified, for example, by the method of genomic walking, using as a
starting point the nucleotide sequence, or fragment thereof, of mouse NRG3 of FIG. 1 (SEQ ID
NO:1), or human NRG3 of FIG. 2 (SEQ ID NO:5) or FIG. 3 (SEQ ID NO:22). DNA encoding the mouse and
human NRG3 polypeptides is deposited with the American Type Culture Collection as ATCC 209156
(mouse; pLXSN.mNRG3), ATCC 209157 (human; pRK5.tk.neo.hNRG3B2), or ATCC 209297 (human;
pRK5.tk.neo.hNRG3).

5. Document ID: US 6117650 A

L12: Entry 5 of 45
File: USPT
Sep 12, 2000

US-PAT-NO: 6117650
DOCUMENT-IDENTIFIER: US 6117650 A
TITLE: Assay for cardiac hypertrophy
DATE-ISSUED: September 12, 2000

US-CL-CURRENT: 435/29; 435/34, 435/40.5

APPL-NO: 8/ 898911
DATE FILED: July 23, 1997

PARENT-CASE:
This application is a continuation of application Ser. No. 08/452,555 filed May 25, 1995, now
abandoned, which is a continuation of application Ser. No. 08/286,304 filed Aug. 5, 1994, now
U.S. Pat. No. 5,571,893, which is a continuation of application Ser. No. 08/233,609 filed Apr.
25, 1994, now U.S. Pat. No. 5,534,615.

IN: King; Kathleen

AB: An assay to test for hypertrophic activity in myocytes is described where wells
are precoated with D-MEM/F-12 and fetal calf serum, plated with myocytes, cultured, and any
change in size of the cells is determined. The growth medium may contain insulin,
transferrin and aprotinin.

L12: Entry 5 of 45
File: USPT
Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117650 A
TITLE: Assay for cardiac hypertrophy

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

6. Document ID: US 6096527 A

L12: Entry 6 of 45
File: USPT
Aug 1, 2000

US-PAT-NO: 6096527
DOCUMENT-IDENTIFIER: US 6096527 A
TITLE: Nucleic acids encoding protein tryosine kinases
DATE-ISSUED: August 1, 2000

US-CL-CURRENT: 435/194; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/69.1, 435/69.7, 536/23.5

APPL-NO: 8/ 445461
DATE FILED: May 22, 1995

PARENT-CASE:
This application is a continuation of U.S. application Ser. No. 08/170,558 filed Dec. 20, 1993,

which is a continuation of U.S. application Ser. No. 08/157,563 filed Nov. 23, 1993 (abandoned),
which applications are incorporated herein by reference and to which applications priority is
claimed under 35 USC .sctn. 120.

IN: Godowski; Paul J., Mark; Melanie R., Scadden; David T.

AB: The protein tyrosine kinase receptors, designated Rse and HPTK6, have been
purified from human and/or murine cell tissues. Rse and HPTK6 have been cloned from a cDNA
library of a human liver carcinoma cell line (i.e., Hep, 3B) using PCR amplification.
Provided herein are nucleic acid sequences encoding Rse and HPTK6 useful as diagnostics and
in the recombinant preparation of Rse and HPTK6. Rse and HPTK6 are used in the preparation
and purification of antibodies thereto and in diagnostic assays.

L12: Entry 6 of 45

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096527 A
TITLE: Nucleic acids encoding protein tryosine kinases

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

7. Document ID: US 6096873 A

L12: Entry 7 of 45

File: USPT

Aug 1, 2000

US-PAT-NO: 6096873
DOCUMENT-IDENTIFIER: US 6096873 A
TITLE: Gamma-heregulin
DATE-ISSUED: August 1, 2000

US-CL-CURRENT: 530/399; 530/300, 530/350, 536/23.5

APPL-NO: 8/ 891845
DATE FILED: July 10, 1997

PARENT-CASE:
RELATED APPLICATIONS This is a non-provisional application filed under 37 CFR 1.53(b)(1),
claiming priority under 35 USC Section 119(e) to provisional Application Ser. No. 60/021,640
filed on Jul. 12, 1996.

IN: Schaefer; Gabriele Maria, Sliwkowski; Mark

AB: A novel member of the heregulin superfamily has been identified which is
designated ".gamma.-HRG". This molecule, secreted by human breast cancer MDA-MB-175 cells,
leads to the formation of a constitutive active receptor complex and stimulates the growth
of these cells in an autocrine manner. .gamma.-HRG polypeptide and nucleic acid are
disclosed, together with various uses therefor (e.g. use of .gamma.-HRG nucleic acid for the
recombinant production of .gamma.-HRG). .gamma.-HRG antagonists (e.g. neutralizing
antibodies and antisense nucleic acid molecules) as well as uses therefor are also
described.

L12: Entry 7 of 45

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096873 A
TITLE: Gamma-heregulin

DEPR:
It is further envisioned that the .gamma.-HRG of this invention may be produced by homologous
recombination, as provided for in WO 91/06667, published May 16, 1991. Briefly, this method
involves transforming a cell containing an endogenous .gamma.-HRG gene with a homologous DNA,
which homologous DNA comprises (a) an amplifiable gene (e.g. a gene encoding dihydrofolate
reductase (DHFR)), and (b) at least one flanking sequence, having a length of at least about 150
base pairs, which is homologous with a nucleotide sequence in the cell genome that is within or
in proximity to the gene encoding .gamma.-HRG. The transformation is carried out under conditions
such that the homologous DNA integrates into the cell genome by recombination. Cells having
integrated the homologous DNA are then subjected to conditions which select for amplification of
the amplifiable gene, whereby the .gamma.-HRG gene is amplified concomitantly. The resulting
cells are then screened for production of desired amounts of .gamma.-HRG. Flanking sequences that
are in proximity to a gene encoding .gamma.-HRG are readily identified, for example, by the
method of genomic walking, using as a starting point the nucleotide sequence of .gamma.-HRG of
FIG. 1.

8. Document ID: US 6096871 A

L12: Entry 8 of 45

File: USPT

Aug 1, 2000

US-PAT-NO: 6096871
DOCUMENT-IDENTIFIER: US 6096871 A
TITLE: Polypeptides altered to contain an epitope from the Fc region of an IgG molecule for increased half-life
DATE-ISSUED: August 1, 2000

US-CL-CURRENT: 530/387.3; 424/133.1, 424/145.1, 435/328, 435/69.6, 435/69.7, 530/388.23

APPL-NO: 8/ 422093
DATE FILED: April 14, 1995

IN: Presta; Leonard G., Snedecor; Bradley R.

AB: Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

L12: Entry 8 of 45
File: USPT
Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096871 A
TITLE: Polypeptides altered to contain an epitope from the Fc region of an IgG molecule for increased half-life

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

9. Document ID: US 6091001 A

L12: Entry 9 of 45
File: USPT
Jul 18, 2000

US-PAT-NO: 6091001
DOCUMENT-IDENTIFIER: US 6091001 A
TITLE: Production of antibodies using Cre-mediated site-specific recombination
DATE-ISSUED: July 18, 2000

US-CL-CURRENT: 800/18; 435/354, 800/21, 800/25, 800/4

APPL-NO: 8/ 412777
DATE FILED: March 29, 1995

IN: Jakobovits; Aya, Zsebo; Krisztina M.

AB: A method to produce a cell expressing an antibody from a genomic sequence of the cell comprising a modified immunoglobulin locus using Cre-mediated site-specific recombination is disclosed. The method involves first transfecting an antibody-producing cell with a homology-targeting vector comprising a lox site and a targeting sequence homologous to a first DNA sequence adjacent to the region of the immunoglobulin loci of the genomic sequence which is to be converted to a modified region, so the first lox site is inserted into the genomic sequence via site-specific homologous recombination. Then the cell is transfected with a lox-targeting vector comprising a second lox site suitable for Cre-mediated recombination with the integrated lox site and a modifying sequence to convert the region of the immunoglobulin loci to the modified region. This conversion is performed by interacting the lox sites with Cre in vivo, so that the modifying sequence inserts into the genomic sequence via Cre-mediated site-specific recombination of the lox sites. Also disclosed are a form of the method used to produce a cell expressing a modified antibody molecule using Cre-mediated site-specific recombination, and antibody-producing cells obtainable by the disclosed methods. Class-switching modifications of human antibodies produced in murine hybridoma cells are exemplified.

L12: Entry 9 of 45
File: USPT
Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6091001 A
TITLE: Production of antibodies using Cre-mediated site-specific recombination

DEPR:
The invention method in Scheme A requires integration of only a single lox site adjacent to the constant region gene to be modified. In this scheme, a first lox site is integrated adjacent to the constant region gene (human C.sub..mu.) which is to be converted to a modified constant region (human C.sub..gamma.), via site-specific homologous recombination with genomic DNA. A vector (comprised of a circular DNA) comprising a second lox site and a selectable marker gene (DHFR) is then transfected into the cells, and the lox sites are transiently interacted with Cre.
Cre is provided by cotransfection of a vector which transiently expresses a cre gene, as described above. Cre-mediated site-specific recombination of the lox site in the genome with the lox site on the circular vector results in insertion into the genome of the modifying sequence (C.sub..gamma.) with the selectable marker gene (DHFR). The desired transfectants, which have two lox sites flanking the DHFR and C.sub..gamma. genes, are stable in the absence of further Cre expression and are obtained by selecting for expression of the DHFR marker gene (i.e. resistance to the drug methotrexate indicated as "Met.sup.R " in FIG. 1). Verification of the desired

modifications of the immunoglobulin loci after each transfection and selection step is provided
by conventional genetic analyses, such as by PCR amplification, as described above.

10. Document ID: US 6087144 A

L12: Entry 10 of 45
File: USPT
Jul 11, 2000

US-PAT-NO: 6087144
DOCUMENT-IDENTIFIER: US 6087144 A
TITLE: Protein tyrosine kinases
DATE-ISSUED: July 11, 2000

US-CL-CURRENT: 435/194; 435/69.1, 514/2, 530/350, 530/395

APPL-NO: 8/ 447314
DATE FILED: May 22, 1995

PARENT-CASE:
CROSS REFERENCES This application is a divisional of co-pending U.S. application Ser. No.
08/170,558 filed Dec. 20, 1993, which application is a continuation of U.S. application Ser. No.
08/157,563 filed Nov. 23, 1993 (abandoned), which applications are incorporated herein by
reference and to which applications priority is claimed under 35 USC sctn.120.

IN: Scadden; David T., Baker; Kevin P., Baron; Will F.

AB: The protein tyrosine kinase receptors, designated Rse and HPTK6, have been
purified from human and/or murine cell tissues. Rse and HPTK6 have been cloned from a cDNA
library of a human liver carcinoma cell line (i.e., Hep 3B) using PCR amplification.
Provided herein are nucleic acid sequences encoding Rse and HPTK6 useful as diagnostics and
in the recombinant preparation of Rse and HPTK6. Rse and HPTK6 are used in the preparation
and purification of antibodies thereto and in diagnostic assays.

L12: Entry 10 of 45
File: USPT
Jul 11, 2000

DOCUMENT-IDENTIFIER: US 6087144 A
TITLE: Protein tyrosine kinases

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

11. Document ID: US 6025157 A

L12: Entry 11 of 45
File: USPT
Feb 15, 2000

US-PAT-NO: 6025157
DOCUMENT-IDENTIFIER: US 6025157 A
TITLE: Neurturin receptor
DATE-ISSUED: February 15, 2000

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 536/23.1, 536/23.5

APPL-NO: 8/ 957063
DATE FILED: October 24, 1997

PARENT-CASE:
RELATED APPLICATIONS This application claims priority to provisional applications U.S. Ser. No.
60/038,839 filed Feb. 18, 1997 and U.S. Ser. No. 60/049,818 filed Jun. 9, 1997, the contents of
which are incorporated herein by reference in their entirety.

IN: Klein; Robert D., Rosenthal; Arnon, Hynes; Mary A.

AB: NTNR.alpha., NTNR.alpha. extracellular domain (ECD), NTNR.alpha. variants,
chimeric NTNR.alpha. (e.g., NTNR.alpha. immunoadhesin), and antibodies which bind thereto
(including agonist and neutralizing antibodies) are disclosed. Various uses for these
molecules are described, including methods to modulate cell activity and survival by
response to NTNR.alpha.-ligands, for example NTN, by providing NTNR.alpha. to the cell.

L12: Entry 11 of 45
File: USPT
Feb 15, 2000

DOCUMENT-IDENTIFIER: US 6025157 A
TITLE: Neurturin receptor

DEPR:
Briefly, this method involves transforming primary human cells containing a NTNR.alpha.-encoding
gene with a construct (i.e., vector) comprising an amplifiable gene (such as dihydrofolate
reductase (DHFR) or others discussed below) and at least one flanking region of a length of at
least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of
the NTNR.alpha. gene to provide amplification of the NTNR.alpha. gene. The amplifiable gene must
be at a site that does not interfere with expression of the NTNR.alpha. gene. The transformation
is conducted such that the construct becomes homologously integrated into the genome of the

primary cells to define an amplifiable region.

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

12. Document ID: US 6020144 A

L12: Entry 12 of 45

File: USPT

Feb 1, 2000

US-PAT-NO: 6020144
DOCUMENT-IDENTIFIER: US 6020144 A
TITLE: Sustained delivery device comprising a Leishmania protozoa and methods of making and using
the same
DATE-ISSUED: February 1, 2000

US-CL-CURRENT: 435/7.22; 424/269.1, 424/94.3, 435/245, 435/258.3, 435/91.41, 435/91.42

APPL-NO: 8/ 735507
DATE FILED: October 23, 1996

PARENT-CASE:
REFERENCE TO RELATED APPLICATIONS The present application claims priority to a provisional patent
application, U.S.S.N. 60/026,006, Attorney Docket No. SYM-001PR, filed on Sep. 12, 1996, the
disclosure of which is incorporated by reference herein .

IN: Gueiros-Filho; Frederico J., Beverley; Stephen M., Vaccaro; Dennis E.

AB: Disclosed herein are novel medical devices, particular well-suited for sustained
delivery of therapeutically-significant substances. Also disclosed are methods of making and
using these delivery devices. Using these devices and methods the present invention teaches
sustained, targeted and reversible delivery of immunostimulating agents, as well as
therapeutic agents such as enzymes, hormones and neurotransmitters, to name but a few.

L12: Entry 12 of 45

File: USPT

Feb 1, 2000

DOCUMENT-IDENTIFIER: US 6020144 A
TITLE: Sustained delivery device comprising a Leishmania protozoa and methods of making and using
the same

DEPR:
DHFR-TS as a negative marker in primary transfections. To test the use of selection against
DHFR-TS in primary transfection experiments, a 7.8-kb BglII targeting fragment derived from
plasmid pR, which contains a deletion of the whole DHFR-TS coding region (Kapler, G. M., C. M.
Coburn, and S. M. Beverley (1990) Mol. Cell Biol. 10:1084-1094, the disclosure of which is herein
incorporated by reference) was used. Homologous replacement at DHFR-TS by this fragment was
expected to yield a deletion of the gene. To exhibit the desired dhfr-ts.sup.- phenotype
following transfection of diploid wild-type cells, two events were required, either two
independent replacements or one replacement combined with LOH.

DEPR:
This method can be used to generate null-targeted deletions at any non-essential genetic locus by
exploiting the fact that DHFR-TS can act both as a positive and a negative marker. In this
example, a given non-essential genetic locus is targeted for deletion. Non-essential, as defined
herein, means that deletion of the locus is not lethal to the cell. In this example, the use of
DHFR-TS as both a positive and a negative marker requires that the cell be dhft-ts.sup.-, and
preferably does not contain any DHFR-TS gene sequence so as to avoid homologous recombination
with the DHFR-TS sequences. In a first step, the cell is transfected with DNA comprising the
DHFR-TS gene flanked by sequences complementary to the sequences flanking the targeted
non-essential gene. Positive selection for DHFR-TS isolates cells having undergone homologous
recombination to replace the target gene with the DHFR-TS gene. The replacement is confirmed
using Southern blotting methods known in the art, and disclosed herein. In a second step, this
cell containing the DHFR-TS gene at the desired genetic locus is transfected with DNA comprising
the same flanking sequences as above, but with no DNA inserted between the flanking sequences.
Negative selection against DHFR-TS, using methods disclosed herein, permits isolation of cells
having lost the DHFR-TS gene due to homologous recombination. These cells are now deleted for the
non-essential target genetic locus such that no DNA sequence replaces the target genetic locus.
The deletion is confirmed using Southern blotting methods known in the art, and disclosed herein.

13. Document ID: US 6015708 A

L12: Entry 13 of 45

File: USPT

Jan 18, 2000

US-PAT-NO: 6015708
DOCUMENT-IDENTIFIER: US 6015708 A
TITLE: Gene manipulation and expression using genomic elements
DATE-ISSUED: January 18, 2000

US-CL-CURRENT: 435/325; 435/254.21, 435/320.1, 435/455, 435/471, 435/70.1

APPL-NO: 8/ 462947
DATE FILED: June 5, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of application Ser.
No. 08/102,567 filed Aug. 5, 1993 (U.S. Pat. No. 5,578,461), which is a continuation of Ser. No.
08/001,898 filed Jan. 7, 1993 (abandoned), which is a continuation of Ser. No. 07/696,216 filed
May 6, 1991 (abandoned) which is a continuation-in-part of application Ser. No. 07/432,069 filed
Nov. 6, 1989 (abandoned), and claims priority to PCT/US90/06425 filed Nov. 6, 1990.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
WO PCT/US90/06425 November 6, 1990

IN: Sherwin; Stephen, Klapholz; Sue, Skoultchi; Arthur

AB: Expression of mammalian target genes is achieved by employing chromosomal target
DNA, either native primary cells or YACs in a yeast host, where the YACs include a fragment
of a mammalian chromosome, the fragment comprising the target gene. Employing homologous
recombination, an amplifiable gene is integrated into the mammalian fragment at a site to
allow for amplification. In the same step, or one or more steps, as desired, the mammalian
gene and/or the transcriptional system may be modified by in vivo mutagenesis. The resulting
construct from homologous recombination may then be transformed into a mammalian expression
host and integrated into the host genome, either randomly or by homologous recombination.
The amplifiable gene may then be amplified by an appropriate agent providing for multiple
copies of the target gene and the expression host grown to provide for high yields of the
desired wild-type or modified protein.

L12: Entry 13 of 45
File: USPT
Jan 18, 2000

DOCUMENT-IDENTIFIER: US 6015708 A
TITLE: Gene manipulation and expression using genomic elements

BSPR:
Mansour et al., Nature, 336:348-352 (1988), describe a general strategy for targeting mutations
to non-selectable genes. Weidle et al., Gene, 66:193-203, (1988), describe amplification of
tissue-type plasminogen activator with a DHFR gene and loss of amplification in the absence of
selective pressure. Murnane and Yezzi, Somatic Cell and Molecular Genetics, 14:273-286, (1988),
describe transformation of a human cell line with an integrated selectable gene marker lacking a
transcriptional promoter, with tandem duplication and amplification of the gene marker. Thomas
and Capecchi, Cell, 51:503-512, (19871, describe site-directed mutagenesis by gene targeting in
mouse embryo-derived stem cells. Song et al., Proc. Natl. Acad. Sci. USA, 84:6820-6824, (1987),
describe homologous recombination in human cells by a two staged integration. Liskay et al.,
"Homologous Recombination Between Repeated Chromosomal Sequences in Mouse Cells," Cold Spring
Harbor, Symp. Quant. Biol. 49:13-189, (1984), describe integration of two different mutations of
the same gene and homologous recombination between the mutant genes. Rubnitz and Subramani, Mol.
and Cell. Biol. 4:2253-2258, (1984), describe the minimum amount of homology required for
homologous recombination in mammalian cells. Kim and Smithies, Nucl. Acids. Res. 16:8887-8903,
(1988), describe an assay for homologous recombination using the polymerase chain reaction.

14. Document ID: US 6001621 A

L12: Entry 14 of 45
File: USPT
Dec 14, 1999

US-PAT-NO: 6001621
DOCUMENT-IDENTIFIER: US 6001621 A
TITLE: Protein tyrosine kinases
DATE-ISSUED: December 14, 1999

US-CL-CURRENT: 435/194; 435/69.1, 435/69.7, 530/350

APPL-NO: 8/ 170558
DATE FILED: December 20, 1993

PARENT-CASE:
This is a continuing application of U.S. Ser. No. 08/157,563, filed Nov. 23, 1993, now abandoned.

IN: Godowski; Paul J., Mark; Melanie R., Scadden; David T.

AB: The protein tyrosine kinase receptors, designated Rse and HPTK6, have been
purified from human and/or murine cell tissues. Rse and HPTK6 have been cloned from a cDNA
library of a human liver carcinoma cell line (i.e., Hep 3B) using PCR amplification.
Provided herein are nucleic acid sequences encoding Rse and HPTK6 useful as diagnostics and
in the recombinant preparation of Rse and HPTK6. Rse and HPTK6 are used in the preparation
and purification of antibodies thereto and in diagnostic assays.

L12: Entry 14 of 45
File: USPT
Dec 14, 1999

DOCUMENT-IDENTIFIER: US 6001621 A
TITLE: Protein tyrosine kinases

DEPR:
Most of the discussion below pertains to production of rPTK by culturing cells transformed with a
vector containing rPTK nucleic acid and recovering the polypeptide from the cell culture. It is
further envisioned that the rPTK of this invention may be produced by homologous recombination,
as provided for in WO 91/06667 published May 16, 1991. Briefly, this method involves transforming
primary mammalian cells containing endogenous rPTK gene (e.g., human

cells if the desired rPTK is
human) with a construct (i.e., vector) comprising an amplifiable gene [such as dihydrofolate
reductase (DHFR) or others discussed below] and at least one flanking region of a length of at
least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of
the rPTK gene to provide amplification of the rPTK gene. The amplifiable gene must be at a site
that does not interfere with expression of the rPTK gene. The transformation is conducted such
that the construct becomes homologously integrated into the genome of the primary cells to define
an amplifiable region.

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

15. Document ID: US 5998144 A

L12: Entry 15 of 45
File: USPT
Dec 7, 1999

US-PAT-NO: 5998144
DOCUMENT-IDENTIFIER: US 5998144 A
TITLE: Method for integrating genes at specific sites in mammalian cells via homologous
recombination and vectors for accomplishing the same
DATE-ISSUED: December 7, 1999

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/455, 435/463, 435/91.41, 536/23.5, 536/23.7,
536/23.72

APPL-NO: 9/ 023715
DATE FILED: February 13, 1998

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of U.S. Ser. No. 08/819,866, U.S.
Pat. No. 5,830,698, filed on Mar. 14, 1997.

IN: Reff; Mitchell R., Barnett; Richard Spence, McLachlan; Karen Retta

AB: A method for achieving site specific integration of a desired DNA at a target
site in a mammalian cell via homologous recombination is described. This method provides for
the reproducible selection of cell lines wherein a desired DNA is integrated at a
predetermined transcriptionally active site previously marked with a marker plasmid. The
method is particularly suitable for the production of mammalian cell lines which secrete
mammalian proteins at high levels, in particular immunoglobulins. Novel vectors and vector
combinations for use in the subject cloning method are also provided.

L12: Entry 15 of 45
File: USPT
Dec 7, 1999

DOCUMENT-IDENTIFIER: US 5998144 A
TITLE: Method for integrating genes at specific sites in mammalian cells via homologous
recombination and vectors for accomplishing the same

BSPR:
The present invention relates to a process of targeting the integration of a desired exogenous
DNA to a specific location within the genome of a mammalian cell. More specifically, the
invention describes a novel method for identifying a transcriptionally active target site ("hot
spot") in the mammalian genome, and inserting a desired DNA at this site via homologous
recombination. The invention also optionally provides the ability for gene amplification of the
desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in
combination with the exogenous DNA. The invention additionally describes the construction of
novel vectors suitable for accomplishing the above, and further provides mammalian cell lines
produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

BSPR:
Unlike most other homologous systems employed in mammalian cells, this system exhibits no
background. Therefore, cells which have only undergone random integration of the vector do not
survive the selection. Thus, any gene of interest cloned into the targeting plasmid is expressed
at high levels from the marked hot spot. Accordingly, the subject method of gene expression
substantially or completely eliminates the problems inherent to systems of random integration,
discussed in detail above. Moreover, this system provides reproducible and high level expression
of any recombinant protein at the same transcriptionally active site in the mammalian genome. In
addition, gene amplification may be effected at this particular transcriptionally active site by
including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

16. Document ID: US 5981214 A

L12: Entry 16 of 45
File: USPT
Nov 9, 1999

US-PAT-NO: 5981214
DOCUMENT-IDENTIFIER: US 5981214 A

TITLE: Production of proteins using homologous recombination
DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 435/69.1; 435/355, 435/357, 435/358, 435/364, 435/365.1, 435/455, 435/463, 435/464, 435/70.1, 536/24.1

APPL-NO: 8/ 467244
DATE FILED: June 6, 1995

PARENT-CASE:
This application is a continuation of application Ser. No. 08/102,390, filed Aug. 5, 1993, pending, which is a continuation of application Ser. No. 07/787,390, filed Nov. 4, 1991 as the national stage of International application PCT/US90/06346 filed Nov. 6, 1990, now abandoned, which is a continuation-in-part of application Ser. No. 432,069, filed Nov. 6, 1989, now abandoned.

IN: Skoultchi; Arthur I.

AB: Methods and compositions are provided for expression of mammalian genes in culture. An amplifiable gene is introduced by homologous recombination in juxtaposition to a target gene, the resulting combination of amplifiable gene and target gene transferred to a convenient host and the target gene amplified by means of the amplifiable gene. The resulting expression host may then be grown in culture with enhanced expression of the target gene.

L12: Entry 16 of 45
File: USPT
Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981214 A
TITLE: Production of proteins using homologous recombination

BSPR:
Mansour et al., Nature, 336:348-352 (1988), describe a general strategy for targeting mutations to non-selectable genes. Weidle et al., Gene, 66:193-203, (1988), describe amplification of tissue-type plasminogen activator with a DHFR gene and loss of amplification in the absence of selective pressure. Murnane and Yezzi, Somatic Cell and Molecular Genetics, 14:273-286, (1988), describe transformation of a human cell line with an integrated selectable gene marker lacking a transcriptional promoter, with tandem duplication and amplification of the gene marker. Thomas and Capecchi, Cell, 51:503-512, (19871, describe site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Song et al., Proc. Natl. Acad. Sci. USA, 84:6820-6824, (1987), describe homologous recombination in human cells by a two staged integration. Liskay et al., "Homologous Recombination Between Repeated Chromosomal Sequences in Mouse Cells," Cold Spring Harbor, Symp. Quant. Biol. 49:13-189, (1984), describe integration of two different mutations of the same gene and homologous recombination between the mutant genes. Rubnitz and Subramani, Mol. and Cell. Biol. 4:2253-2258, (1984), describe the minimum amount of homology required for homologous recombination in mammalian cells. Kim and Smithies, Nucl. Acids. Res. 16:8887-8903, (1988), describe an assay for homologous recombination using the polymerase chain reaction.

DEPR:
The plasmid pCG linearized with NotI is introduced into the primary recipients by electroporation employing DNA at 10 nM. The resulting cells are then grown in selective medium (EEMEM with 200 .mu.g/ml hygromycin B). Resistant colonies are isolated and analyzed by PCR (Kim and Smithies, Nucleic Acids Res. 16:8887-8903 (1988)) using as primers the sequences (SEQ ID NO:2) GCGGCCTCGGCCTCTGCATA and (SEQ ID NO:3) CATCTCCCCTCTGGAGTGGA to distinguish homologous integrants from random ones. Amplification of cellular DNA by PCR using these two primers yields a fragment of 1.9 kb only when DNA from correctly targeted cells is present. Cells comprising the DHFR gene integrated into the t-PA region are expanded and used as a source of genetic material for preparation of secondary recipients.

17. Document ID: US 5968502 A

L12: Entry 17 of 45
File: USPT
Oct 19, 1999

US-PAT-NO: 5968502
DOCUMENT-IDENTIFIER: US 5968502 A
TITLE: Protein production and protein delivery
DATE-ISSUED: October 19, 1999

US-CL-CURRENT: 424/93.21; 424/425, 435/320.1, 435/325, 435/455, 435/69.1, 536/23.1

APPL-NO: 8/ 451894
DATE FILED: May 26, 1995

PARENT-CASE:
RELATED APPLICATION This application is a division of application Ser. No. 07/985.586 filed Dec. 3, 1992, abandoned, which is a continuation-in-part of U.S. patent application. Ser. No. 07/789,188, filed on Nov. 5, 1991, abandoned, and is also a continuation-in-part of U.S. patent application Ser. No. 07/911.533 filed on Jul. 10, 1992, abandoned, and is also a continuation-in-part of U.S. patent application Ser. No. 07/787,840, filed on Nov. 5, 1991, abandoned. All of the above applications are incorporated herein by reference in their entirety.

IN: Treco; Douglas, Heartlein; Michael W., Selden; Richard F

AB: The present invention relates to transfected primary, secondary, and immortalized cells of vertebrate origin particularly mammalian origin, transfected with exogenous genetic material (DNA) which encodes a desired (e.g., a therapeutic) product or is itself a desired (e.g., therapeutic) product, methods by which primary, secondary and immortalized cells are transfected to include exogenous genetic material, including DNA targeting by homologous recombination, methods for the activation and amplification of endogenous cellular genes, methods by which cells useful for large-scale protein production can be obtained, methods of producing clonal cell strains or heterogenous cell strains, and methods of gene therapy in which transfected primary, secondary or immortalized cells are used., The present invention

includes primary, secondary, and immortalized cells, such as fibroblasts, keratinocytes,
epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the
blood, muscle cells, and other cells which can be cultured.

L12: Entry 17 of 45

File: USPT

Oct 19, 1999

DOCUMENT-IDENTIFIER: US 5968502 A
TITLE: Protein production and protein delivery

DEPR:
The use of homologous recombination to target a gene of therapeutic interest to a specific
position in a cell's genomic DNA can be expanded upon and made more useful for producing products
for therapeutic purposes (e.g., pharmaceutics, gene therapy) by the insertion of a gene through
which cells containing amplified copies of the gene can be selected for by exposure of the cells
to an appropriate drug selection regimen. For example, pE3neo/hGH (Example 1d) can be modified by
inserting the dhfr, ada, or CAD gene at a position immediately adjacent to the hGH or neo genes
in pE3neo/hGH. Primary, secondary, or immortalized cells are transfected with such a plasmid and
correctly targeted events are identified. These cells are further treated with increasing
concentrations of drugs appropriate for the selection of cells containing amplified genes (for
dhfr, the selective agent is methotrexate, for CAD the selective agent is
N-(phosphonacetyl)-L-aspartate (PALA), and for ada the selective agent is an adenine nucleoside
(e.g., alanosine). In this manner the integration of the gene of therapeutic interest will be
coamplified along with the gene for which amplified copies are selected. Thus, the genetic
engineering of cells to produce genes for therapeutic uses can be readily controlled by
preselecting the site at which the targeting construct integrates and at which the amplified
copies reside in the amplified cells.

18. Document ID: US 5955333 A

L12: Entry 18 of 45

File: USPT

Sep 21, 1999

US-PAT-NO: 5955333
DOCUMENT-IDENTIFIER: US 5955333 A
TITLE: Double targeted gene replacement in unicellular diploid organisms
DATE-ISSUED: September 21, 1999

US-CL-CURRENT: 435/471; 435/254.11, 435/258.3, 435/477

APPL-NO: 8/ 762529
DATE FILED: December 9, 1996

PARENT-CASE:
RELATED APPLICATIONS This is a continuation of application Ser. No. 08/489,701 filed on Jun. 13,
1995, now abandoned, which is a continuation of application Ser. No. 08/066,718 filed on May 24,
1993, now abandoned, which is a continuation-in-part of application Ser. No. 07/744,313 filed on
Aug. 13, 1991, now abandoned.

IN: Beverley; Stephen M., Cruz; Angela K.

AB: Homozygous gene replacement can be created in unicellular diploid organisms by
individually targeting each allele of a gene with genetic constructs containing two
different and independent selectable markers. Selection for both markers indicates
replacement of both alleles of the gene, or portion thereof. The method can be used to study
gene function in these organisms and to create mutant organisms such as attenuated strains
of parasitic protozoans for use in live vaccines.

L12: Entry 18 of 45

File: USPT

Sep 21, 1999

DOCUMENT-IDENTIFIER: US 5955333 A
TITLE: Double targeted gene replacement in unicellular diploid organisms

DEPR:
To test whether HYG was suitable as a marker for gene replacement, we transfected the 3.3 kb
SalI-SmaI fragment of pX63-HYG (FIGS. 1A, 1B). This fragment contains only 0.9 and 1.4 kb of 5'
and 3' DNA which normally flanks the L. major dhfr-ts coding region, whereas a previously
utilized targeting fragment contained 5 and 2 kb respectively of dhfr-ts flanking DNA (Cruz, A.
and Beverley, S. M. (1990) Nature 348: 171-174). We utilized less than 5 .mu.g of the targeting
DNA fragment, since simple replacement events in Leishmania are favored by relatively low DNA
amounts (Cruz, A. and Beverley, S. M. (1990) Nature 348:171-174). 2-4 colonies/.mu.g targeting
fragment were obtained, 5-10% of the yield obtained with circular pX63-HYG. A similar result was
obtained with the analogous 3.3 kb fragment from pX (containing the NEO marker) after plating on
G418-containing media. In contrast, the larger NEO targeting fragment yielded colonies at 20-100%
the efficiency of the circular controls (Cruz, A. and Beverley, S. M. (1990) Nature 348:171-174),
consistent with studies in other species showing increased targeting frequencies with fragments
bearing longer homologous sequences (Shulman, M. J. et al. (1990) Mol. Cell. Biol. 10:4466-4472.;
Thomas, K. R. and Capecchi, M. R. (1987) Cell 51:503-512).

DEPR:
Although the relative frequency of homologous gene replacement in Leishmania is high, the fact
that Leishmania is a functionally asexual diploid requires that both alleles be modified prior to
functional testing. The availability of a second selectable marker allowed us to overcome this
problem. Null mutants at dhfr-ts were obtained by two rounds of gene targeting, first with
NEO-containing and then with HYG-containing targeting vectors (the order is unimportant;
unpublished data). The efficiency of each step was comparable. Although previous studies
suggested that it should be possible to obtain simultaneous replacement of both dhfr-ts alleles
(Cruz, A. and Beverley, S. M. (1990) Nature 348:171-174), we have not been able to accomplish
this by transfection of the NEO and HYG targeting fragments simultaneously. We presume that this
reflects the low cumulative frequency expected for simultaneous transfection and replacement.
Improved procedures may remove this limitation at the dhfr-ts locus in the

future, however for
loci in which the viability of the null mutant is unknown the two step procedure may be
preferable.

19. Document ID: US 5869046 A

L12: Entry 19 of 45

File: USPT

Feb 9, 1999

US-PAT-NO: 5869046
DOCUMENT-IDENTIFIER: US 5869046 A
TITLE: Altered polypeptides with increased half-life
DATE-ISSUED: February 9, 1999

US-CL-CURRENT: 424/133.1; 424/153.1, 530/387.3, 530/388.15, 530/388.7, 530/391.1

APPL-NO: 8/ 422092
DATE FILED: April 14, 1995

IN: Presta; Leonard G., Snedecor; Bradley R.

AB: Polypeptides that are cleared from the kidney and do not contain in their
original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding
epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

L12: Entry 19 of 45

File: USPT

Feb 9, 1999

DOCUMENT-IDENTIFIER: US 5869046 A
TITLE: Altered polypeptides with increased half-life

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

20. Document ID: US 5864020 A

L12: Entry 20 of 45

File: USPT

Jan 26, 1999

US-PAT-NO: 5864020
DOCUMENT-IDENTIFIER: US 5864020 A
TITLE: HTK ligand
DATE-ISSUED: January 26, 1999

US-CL-CURRENT: 530/388.24; 435/188, 530/387.1, 530/391.1, 530/391.3

APPL-NO: 8/ 436054
DATE FILED: May 5, 1995

PARENT-CASE:
CROSS REFERENCES This application is a divisional of co-pending U.S. application Ser. No.
08/277,722 filed 20 Jul. 1994, which application is incorporated herein by reference and to which
application priority is claimed under 35 USC .sctn.120.

IN: Bennett; Brian D., Matthews; William

AB: A novel hepatoma transmembrane kinase receptor ligand (Htk ligand) which binds
to, and activates, the Htk receptor is disclosed. As examples, mouse and human Htk ligands
have been identified in a variety of tissues using a soluble Htk-Fc fusion protein. The
ligands have been cloned and sequenced. The invention also relates to nucleic acids encoding
the ligand, methods for production and use of the ligand, and antibodies directed thereto.

L12: Entry 20 of 45

File: USPT

Jan 26, 1999

DOCUMENT-IDENTIFIER: US 5864020 A
TITLE: HTK ligand

DEPR:
Briefly, this method involves transforming primary mammalian cells containing endogenous Htk
ligand gene (e.g., human cells if the desired Htk ligand is human) with a construct (i.e.,
vector) comprising an amplifiable gene [such as dihydrofolate reductase (DHFR) or others
discussed below] and at least one flanking region of a length of at least about 150 bp that is
homologous with a DNA is sequence at the locus of the coding region of the Htk ligand gene to
provide amplification of the Htk ligand gene. The amplifiable gene must be at a site that does
not,interfere with expression of the Htk ligand gene. The transformation is conducted such that
the construct becomes homologously integrated into the genome of the primary cells to define an
amplifiable region.

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR

and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

21. Document ID: US 5830698 A

L12: Entry 21 of 45

File: USPT

Nov 3, 1998

US-PAT-NO: 5830698
DOCUMENT-IDENTIFIER: US 5830698 A
TITLE: Method for integrating genes at specific sites in mammalian cells via homologous
recombination and vectors for accomplishing the same
DATE-ISSUED: November 3, 1998

US-CL-CURRENT: 435/69.1; 435/320.1, 435/463, 435/465, 536/23.2

APPL-NO: 8/ 819866
DATE FILED: March 14, 1997

IN: Reff; Mitchell E., Barnett; Richard Spence, McLachlan; Karen Retta

AB: A method for achieving site specific integration of a desired DNA at a target
site in a mammalian cell via homologous recombination is described. This method provides for
the reproducible selection of cell lines wherein a desired DNA is integrated at a
predetermined transcriptionally active site previously marked with a marker plasmid. The
method is particularly suitable for the production of mammalian cell lines which secrete
mammalian proteins at high levels, in particular immunoglobulins. Novel vectors and vector
combinations for use in the subject cloning method are also provided.

L12: Entry 21 of 45

File: USPT

Nov 3, 1998

DOCUMENT-IDENTIFIER: US 5830698 A
TITLE: Method for integrating genes at specific sites in mammalian cells via homologous
recombination and vectors for accomplishing the same

BSPR:
The present invention relates to a process of targeting the integration of a desired exogenous
DNA to a specific location within the genome of a mammalian cell. More specifically, the
invention describes a novel method for identifying a transcriptionally active target site ("hot
spot") in the mammalian genome, and inserting a desired DNA at this site via homologous
recombination. The invention also optionally provides the ability for gene amplification of the
desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in
combination with the exogenous DNA. The invention additionally describes the construction of
novel vectors suitable for accomplishing the above, and further provides mammalian cell lines
produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

BSPR:
Unlike most other homologous systems employed in mammalian cells, this system exhibits no
background. Therefore, cells which have only undergone random integration of the vector do not
survive the selection. Thus, any gene of interest cloned into the targeting plasmid is expressed
at high levels from the marked hot spot. Accordingly, the subject method of gene expression
substantially or completely eliminates the problems inherent to systems of random integration,
discussed in detail above. Moreover, this system provides reproducible and high level expression
of any recombinant protein at the same transcriptionally active site in the mammalian genome. In
addition, gene amplification may be effected at this particular transcriptionally active site by
including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

22. Document ID: US 5798448 A

L12: Entry 22 of 45

File: USPT

Aug 25, 1998

US-PAT-NO: 5798448
DOCUMENT-IDENTIFIER: US 5798448 A
TITLE: AL-1 neurotrophic factor antibodies
DATE-ISSUED: August 25, 1998

US-CL-CURRENT: 530/387.1; 424/130.1, 424/132.1, 424/133.1, 424/134.1, 424/135.1, 424/136.1,
424/9.34, 435/7.1, 435/7.2, 435/7.9, 436/512, 436/514, 436/517, 436/518, 436/536, 436/538,
436/547, 436/548, 530/300, 530/350, 530/387.3, 530/387.9, 530/388.1, 530/388.15, 530/388.24,
530/389.1

APPL-NO: 8/ 440815
DATE FILED: May 15, 1995

PARENT-CASE:
This is a continuation of co-pending application Ser. No. 08/330,128 filed on Oct. 27, 1994which
application is incorporated herein by reference and to which application priority is claimed
under 35 USC .sctn. 120.

IN: Caras; Ingrid W., Winslow; John W.

AB: The present invention provides nucleic acids encoding AL-1 protein, as well as
AL-1 protein produced by recombinant DNA methods. Such AL-1 protein is useful in preparing
antibodies and in diagnosing and treating various neuronal disorders.

L12: Entry 22 of 45

File: USPT

Aug 25, 1998

DOCUMENT-IDENTIFIER: US 5798448 A
TITLE: AL-1 neurotrophic factor antibodies

DEPR:
It is further contemplated that the AL-1 of this invention may be produced by homologous
recombination, for example, as described in PCT Pat. Pub. No. WO 91/06667 (published May 16,
1991). Briefly, this method involves transforming cells containing an endogenous gene encoding
AL-1 with a homologous DNA, which homologous DNA comprises (1) an amplifiable gene, such as DHFR,
and (2) at least one flanking sequence, having a length of at least about 150 base pairs, which
is homologous with a nucleotide sequence in the cell genome that is within or in proximity to the
gene encoding AL-1. The transformation is carried out under conditions such that the homologous
DNA integrates into the cell genome by recombination. Cells having integrated the homologous DNA
then are subjected to conditions which select for amplification of the amplifiable gene, whereby
the AL-1 gene amplified concomitantly. The resulting cells then are screened for production of
desired amounts of AL-1. Flanking sequences that are in proximity to a gene encoding AL-1 are
readily identified, for example, by the method of genomic walking, using as a starting point the
AL-1 nucleotide sequence set forth in FIG. 2 (SEQ. ID. NO: 3). Spoerel, et al., Meth. Enz.
152:598-603 (1987).

23. Document ID: US 5773257 A

L12: Entry 23 of 45

File: USPT

Jun 30, 1998

US-PAT-NO: 5773257
DOCUMENT-IDENTIFIER: US 5773257 A
TITLE: Method for producing primed nucleic acid templates
DATE-ISSUED: June 30, 1998

US-CL-CURRENT: 435/91.1; 435/91.2, 530/350

APPL-NO: 8/ 469564
DATE FILED: June 6, 1995

PARENT-CASE:
This is a division of application Ser. No. 08/224,981, filed Apr. 7, 1994 now U.S. Pat. No.
5,646,019; which is a continuation application of Ser. No. 07/425,867, filed Oct. 24, 1989, now
abandoned.

IN: Nielson; Kirk B., Mathur; Eric J.

AB: The present invention relates to an improved method for producing primed nucleic
acid templates. Specifically, it relates to a method, compositions and kits therefor, of
increasing the specificity of primer extension reactions by hybridizing primer to template
in the presence of single-stranded nucleic acid binding protein.

L12: Entry 23 of 45

File: USPT

Jun 30, 1998

DOCUMENT-IDENTIFIER: US 5773257 A
TITLE: Method for producing primed nucleic acid templates

DEPR:
The two primer pairs have sequences that were derived from the human dihydrofolate reductase gene
(DHFR), at exon 1 (pr 1A and pr 1B), or at exon 2 (pr 2A and 2B). However, each primer has 18
nucleotide residues at their 5' ends that are not homologous to the DHFR gene, but rather
correspond in sequence to portions of a universal lambda primer. At the 3' end of each primer
there is a stretch of 33 nucleotides (pr 1A), 27 nucleotides (pr 1B), 27 nucleotides (pr 2A), or
30 nucleotides (pr 2B) that correspond to portions of the human DHFR gene. Thus, when
specifically hybridized to the human genomic DNA DHFR gene, the duplex containing these primers
has a 5' "tail" region comprising 18 nucleotides that is not complementary or hybridized to the
template and is attached to the remaining 3' portion comprising 27 to 33 nucleotides that is
hybridized to the DHFR gene. The above DHFR primers are therefore examples of primers having
substantial complementarity with the target sequence.

24. Document ID: US 5770414 A

L12: Entry 24 of 45

File: USPT

Jun 23, 1998

US-PAT-NO: 5770414
DOCUMENT-IDENTIFIER: US 5770414 A
TITLE: Regulatable retrovirus system for genetic modification of cells
DATE-ISSUED: June 23, 1998

US-CL-CURRENT: 435/456; 435/320.1, 435/353, 435/357

APPL-NO: 8/ 602203
DATE FILED: February 20, 1996

IN: Gage; Fred H., Ray; Jasodhara, Hoshimaru; Minoru

AB: A novel regulatable retroviral vector in which the v-myc oncogene is driven by a
tetracycline-controlled transactivator and a human cytomegalovirus minimal promoter fused to
tet operator sequence useful for immortalization of adult neuronal progenitor cells is
provided. Regulation of a heterologous Producer cell lines which produce high titers of the
recombinant retrovirus are also provided.

L12: Entry 24 of 45

File: USPT

Jun 23, 1998

DOCUMENT-IDENTIFIER: US 5770414 A
TITLE: Regulatable retrovirus system for genetic modification of cells

DEPR:
The promoter in operable linkage with the heterologous gene can be any viral or housekeeping gene
promoter, including for example, cytomegalovirus (CMV) LTR, DHFR, SV40 immediate early gene,
Moloney murine leukemia virus and Rous Sarcoma virus LTR promoter. The promoter sequence may be
homologous or heterologous to the nucleic acid sequence. A wide range of promoters may be
utilized, including viral or tissue specific promoter. Cell or tissue specific promoters can be
utilized to target expression of gene sequences in specific cell populations. Suitable mammalian
and viral promoters for the present invention are available in the art. However, the promoter
operably linked with the heterologous gene must be responsive to the regulatable transactivating
element.

25. Document ID: US 5759775 A

L12: Entry 25 of 45

File: USPT

Jun 2, 1998

US-PAT-NO: 5759775
DOCUMENT-IDENTIFIER: US 5759775 A
TITLE: Methods for detecting nucleic acids encoding AL--1 neurotrophic factor
DATE-ISSUED: June 2, 1998

US-CL-CURRENT: 435/6; 435/91.2, 536/23.5, 536/24.31, 536/24.33

APPL-NO: 8/ 442248
DATE FILED: May 15, 1995

PARENT-CASE:
This is a continuation of co-pending application Ser. No. 08/330,128 filed on Oct. 27, 1994,
which application is incorporated herein by reference and to which application priority is
claimed under 35 USC .sctn. 120.

IN: Caras; Ingrid W., Winslow; John W.

AB: Provided are nucleic acids encoding AL-1 protein, as well as AL-1 protein
produced by recombinant DNA methods. Such AL-1 protein is useful in preparing antibodies and
in diagnosing and treating various neuronal disorders. The present invention provides
methods to preferentially detect or amplify AL-1 nucleic acid in a sample using AL-1
nucleotide sequence probes.

L12: Entry 25 of 45

File: USPT

Jun 2, 1998

DOCUMENT-IDENTIFIER: US 5759775 A
TITLE: Methods for detecting nucleic acids encoding AL--1 neurotrophic factor

DEPR:
It is further contemplated that the AL-1 of this invention may be produced by homologous
recombination, for example, as described in PCT Pat. Pub. No. WO 91/06667 (published May 16,
1991). Briefly, this method involves transforming cells containing an endogenous gene encoding
AL-1 with a homologous DNA, which homologous DNA comprises (1) an amplifiable gene, such as DHFR,
and (2) at least one flanking sequence, having a length of at least about 150 base pairs, which
is homologous with a nucleotide sequence in the cell genome that is within or in proximity to the
gene encoding AL-1. The transformation is carried out under conditions such that the homologous
DNA integrates into the cell genome by recombination. Cells having integrated the homologous DNA
then are subjected to conditions which select for amplification of the amplifiable gene, whereby
the AL-1 gene amplified concomitantly. The resulting cells then are screened for production of
desired amounts of AL-1. Flanking sequences that are in proximity to a gene encoding AL-1 are
readily identified, for example, by the method of genomic walking, using as a starting point the
AL-1 nucleotide sequence set forth in FIG. 2. Spoerel, et al., Meth. Enz. 152:598-603 (1987).

26. Document ID: US 5747035 A

L12: Entry 26 of 45

File: USPT

May 5, 1998

US-PAT-NO: 5747035
DOCUMENT-IDENTIFIER: US 5747035 A
TITLE: Polypeptides with increased half-life for use in treating disorders involving the LFA-1
receptor
DATE-ISSUED: May 5, 1998

US-CL-CURRENT: 424/144.1; 424/130.1, 424/133.1, 424/135.1, 424/141.1, 424/143.1, 424/153.1,
424/154.1, 424/173.1, 514/2 , 514/8, 514/885, 530/387.1

APPL-NO: 8/ 422091
DATE FILED: April 14, 1995

IN: Presta; Leonard G., Snedecor; Bradley R.

AB: Polypeptides that are cleared from the kidney and do not contain in their
original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding
epitope of an Fc region of an IgG and thereby have increased circulatory half-life. Methods
are described herein which utilize these polypeptides in treating disorders involving the
LFA-1 receptor. In one of the described methods of treatment, the polypeptide includes the
amino acid sequence PKNSSMISNTP (SEQ ID NO:3) and may also include the sequence selected
from the group consisting of HQNLSDGK (SEQ ID NO: 1), HQNISDGK (SEQ ID NO:2), HQSLGTQ (SEQ
ID NO:11) and VISSHLGQ (SEQ ID NO:31).

L12: Entry 26 of 45

File: USPT

May 5, 1998

DOCUMENT-IDENTIFIER: US 5747035 A
TITLE: Polypeptides with increased half-life for use in treating disorders

involving the LFA-1
receptor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

27. Document ID: US 5739277 A

L12: Entry 27 of 45
File: USPT
Apr 14, 1998

US-PAT-NO: 5739277
DOCUMENT-IDENTIFIER: US 5739277 A
TITLE: Altered polypeptides with increased half-life
DATE-ISSUED: April 14, 1998

US-CL-CURRENT: 530/326; 530/300, 530/350, 530/387.1

APPL-NO: 8/ 422101
DATE FILED: April 14, 1995

IN: Presta; Leonard G., Snedecor; Bradley R.

AB: Polypeptides that are cleared from the kidney and do not contain in their
original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding
epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

L12: Entry 27 of 45
File: USPT
Apr 14, 1998

DOCUMENT-IDENTIFIER: US 5739277 A
TITLE: Altered polypeptides with increased half-life

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

28. Document ID: US 5733761 A

L12: Entry 28 of 45
File: USPT
Mar 31, 1998

US-PAT-NO: 5733761
DOCUMENT-IDENTIFIER: US 5733761 A
TITLE: Protein production and protein delivery
DATE-ISSUED: March 31, 1998

US-CL-CURRENT: 435/463; 435/69.4, 536/23.51, 536/24.1

APPL-NO: 8/ 451893
DATE FILED: May 26, 1995

PARENT-CASE:
RELATED APPLICATION This application is a continuation of application Ser. No. 07/985,586 filed
Dec. 3, 1992, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No.
07/789,188, filed on Nov. 5, 1991, now abandoned, and is also a continuation-in-part of U.S.
patent application Ser. No. 07/911,533, filed on Jul. 10, 1992, now abandoned, and is also a
continuation-in-part of U.S. patent application Ser. No. 07/787,840, filed on Nov. 5, 1991, now
abandoned. All of the above applications are incorporated herein by reference in their entirety.

IN: Treco; Douglas, Heartlein; Michael W., Selden; Richard F.

AB: The present invention relates to transfected primary, secondary and immortalized
cells of vertebrate origin, particularly mammalian origin, transfected with exogenous
genetic material (DNA) which encodes a desired (e.g., a therapeutic) product or is itself a
desired (e.g., therapeutic) product, methods by which primary, secondary and immortalized
cells are transfected to include exogenous genetic material, including DNA targeting by
homologous recombination, methods for the activation and amplification of endogenous
cellular genes, methods by which cells useful for large-scale protein production can be
obtained, methods of producing clonal cell strains or heterogenous cell strains, and methods
of gene therapy in which transfected primary, secondary or immortalized cells are used. The
present invention includes primary, secondary and immortalized cells, such as fibroblasts,
keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells,

formed
elements of the blood, muscle cells, and other cells which can be cultured.

L12: Entry 28 of 45
File: USPT
Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733761 A
TITLE: Protein production and protein delivery

DEPR:
The use of homologous recombination to target a gene of therapeutic interest to a specific
position in a cell's genomic DNA can be expanded upon and made more useful for producing products
for therapeutic purposes (e.g., pharmaceutics, gene therapy) by the insertion of a gene through
which cells containing amplified copies of the gene can be selected for by exposure of the cells
to an appropriate drug selection regimen. For example, pE3neo/hGH (Example 1d) can be modified by
inserting the dhfr, ada, or CAD gene at a position immediately adjacent to the hGH or neo genes
in pE3neo/hGH. Primary, secondary, or immortalized cells are transfected with such a plasmid and
correctly targeted events are identified. These cells are further treated with increasing
concentrations of drugs appropriate for the selection of cells containing amplified genes (for
dhfr, the selective agent is methotrexate, for CAD the selective agent is
N-(phosphonacetyl)-L-aspartate (PALA), and for ada the selective agent is an adenine nucleoside
(e.g., alanosine). In this manner the integration of the gene of therapeutic interest will be
coamplified along with the gene for which amplified copies are selected. Thus, the genetic
engineering of cells to produce genes for therapeutic uses can be readily controlled by
preselecting the site at which the targeting construct integrates and at which the amplified
copies reside in the amplified cells.

29. Document ID: US 5723585 A

L12: Entry 29 of 45
File: USPT
Mar 3, 1998

US-PAT-NO: 5723585
DOCUMENT-IDENTIFIER: US 5723585 A
TITLE: Method of purifying cardiac hypertrophy factor
DATE-ISSUED: March 3, 1998

US-CL-CURRENT: 530/413; 530/350, 530/380, 930/140

APPL-NO: 8/ 443130
DATE FILED: May 17, 1995

PARENT-CASE:
This is a divisional of application Ser. No. 08/286,304 filed Aug. 5, 1994 now U.S. Pat. No.
5,571,893, which is a continuation-in-part of Ser. No. 08/233,609 filed Apr. 25, 1994, now U.S.
Pat. No. 5,534,615 which application is incorporated herein by reference and to which application
priority is claimed under 35 USC .sctn.120.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CHF, isolated DNA encoding CHF, recombinant or synthetic methods of
preparing CHF, and a method of purifying CHF are disclosed. These CHF molecules are shown to
influence hypertrophic activity and neurological activity. Accordingly, these compounds or
their antagonists may be used for treatment of heart failure, arrhythmic disorders,
inotropic disorders, and neurological disorders.

L12: Entry 29 of 45
File: USPT
Mar 3, 1998

DOCUMENT-IDENTIFIER: US 5723585 A
TITLE: Method of purifying cardiac hypertrophy factor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

30. Document ID: US 5709858 A

L12: Entry 30 of 45
File: USPT
Jan 20, 1998

US-PAT-NO: 5709858
DOCUMENT-IDENTIFIER: US 5709858 A
TITLE: Antibodies specific for Rse receptor protein tyrosine kinase
DATE-ISSUED: January 20, 1998

US-CL-CURRENT: 424/143.1; 424/139.1, 435/7.4, 530/387.3, 530/387.9, 530/388.22, 530/391.1,
530/391.3

APPL-NO: 8/ 445640
DATE FILED: May 22, 1995

PARENT-CASE:
CROSS REFERENCES This application is a continuation of co-pending U.S. application Ser. No.
08/170,558 filed 20 Dec. 1993, pending, which application is a continuation of U.S. application
Ser. No. 08/157,563 filed 23 Nov. 1993 (abandoned), which applications

are incorporated herein by
reference and to which applications priority is claimed under 35 USC .sctn.120.

IN: Godowski; Paul J., Mark; Melanie R., Scadden; David T.

AB: The protein tyrosine kinase receptors, designated Rse and HPTK6, have been
purified from human and/or murine cell tissues. Rse and HPTK6 have been cloned from a cDNA
library of a human liver carcinoma cell line (i.e., Hep 3B) using PCR amplification.
Provided herein are nucleic acid sequences encoding Rse and HPTK6 useful as diagnostics and
in the recombinant preparation of Rse and HPTK6. Rse and HPTK6 are used in the preparation
and purification of antibodies thereto and in diagnostic assays.

L12: Entry 30 of 45
File: USPT
Jan 20, 1998

DOCUMENT-IDENTIFIER: US 5709858 A
TITLE: Antibodies specific for Rse receptor protein tyrosine kinase

DEPR:
Most of the discussion below pertains to production of rPTK by culturing cells transformed with a
vector containing rPTK nucleic acid and recovering the polypeptide from the cell culture. It is
further envisioned that the rPTK of this invention may be produced by homologous recombination,
as provided for in WO 91/06667 published 16 May 1991. Briefly, this method involves transforming
primary mammalian cells containing endogenous rPTK gene (e.g., human cells if the desired rPTK is
human) with a construct (i.e., vector) comprising an amplifiable gene [such as dihydrofolate
reductase (DHFR) or others discussed below] and at least one flanking region of a length of at
least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of
the rPTK gene to provide amplification of the rPTK gene. The amplifiable gene must be at a site
that does not interfere with expression of the rPTK gene. The transformation is conducted such
that the construct becomes homologously integrated into the genome of the primary cells to define
an amplifiable region.

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

31. Document ID: US 5679545 A

L12: Entry 31 of 45
File: USPT
Oct 21, 1997

US-PAT-NO: 5679545
DOCUMENT-IDENTIFIER: US 5679545 A
TITLE: Gene encoding cardiac hypertrophy factor
DATE-ISSUED: October 21, 1997

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/325, 536/23.5

APPL-NO: 8/ 443952
DATE FILED: May 17, 1995

PARENT-CASE:
This is a divisional of application Ser. No. 08/286,304 Aug. 5, 1994, issued as U.S. Pat. No.
5,571,893 on Nov. 5, 1996, which is a continuation-in-part of Ser. No. 08/233,609 filed Apr. 25,
1994, issued U.S. Pat. No. 5,534,615 on Jul. 9, 1996, which application is incorporated herein by
reference and to which application priority is claimed under 35 USC .sctn.120.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CT-1, isolated DNA encoding CT-1, and recombinant or synthetic methods
of preparing CT-1 are disclosed. These CT-1 molecules are shown to influence hypertrophic
activity and neurological activity. Accordingly, these compounds or their antagonists may be
used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and
neurological disorders.

L12: Entry 31 of 45
File: USPT
Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679545 A
TITLE: Gene encoding cardiac hypertrophy factor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation

described below.

32. Document ID: US 5646019 A

L12: Entry 32 of 45

File: USPT

Jul 8, 1997

US-PAT-NO: 5646019
DOCUMENT-IDENTIFIER: US 5646019 A
TITLE: Method for producing primed nucleic acid templates
DATE-ISSUED: July 8, 1997

US-CL-CURRENT: 435/91.5; 435/91.1

APPL-NO: 8/ 224981
DATE FILED: April 7, 1994

PARENT-CASE:
DESCRIPTION This is a continuation of application Ser. No. 07/425,867, filed Oct. 24, 1989, now abandoned.

IN: Nielson; Kirk B., Mathur; Eric J.

AB: The present invention relates to an improved method for producing primed nucleic acid templates. Specifically, it relates to a method, compositions and kits therefor, of increasing the specificity of primer extension reactions by hybridizing primer to template in the presence of single-stranded nucleic acid binding protein.

L12: Entry 32 of 45

File: USPT

Jul 8, 1997

DOCUMENT-IDENTIFIER: US 5646019 A
TITLE: Method for producing primed nucleic acid templates

DEPR:
The two primer pairs have sequences that were derived from the human dihydrofolate reductase gene (DHFR), at exon 1 (pr 1A and pr 1B), or at exon 2 (pr 2A and 2B). However, each primer has 18 nucleotide residues at their 5' ends that are not homologous to the DHFR gene, but rather correspond in sequence to portions of a universal lambda primer. At the 3' end of each primer there is a stretch of 33 nucleotides (pr 1A), 27 nucleotides (pr 1B), 27 nucleotides (pr 2A), or 30 nucleotides (pr 2B) that correspond to portions of the human DHFR gene. Thus, when specifically hybridized to the human genomic DNA DHFR gene, the duplex containing these primers has a 5' "tail" region comprising 18 nucleotides that is not complementary or hybridized to the template and is attached to the remaining 3' portion comprising 27 to 33 nucleotides that is hybridized to the DHFR gene. The above DHFR primers are therefore examples of primers having substantial complementarity with the target sequence.

33. Document ID: US 5641670 A

L12: Entry 33 of 45

File: USPT

Jun 24, 1997

US-PAT-NO: 5641670
DOCUMENT-IDENTIFIER: US 5641670 A
TITLE: Protein production and protein delivery
DATE-ISSUED: June 24, 1997

US-CL-CURRENT: 435/325; 435/254.11, 435/320.1, 435/326, 435/366, 435/367, 435/371, 435/372, 435/372.2, 435/372.3, 435/419

APPL-NO: 8/ 243391
DATE FILED: May 13, 1994

PARENT-CASE:
RELATED APPLICATIONS This application is a Continuation-In-Part of U.S. patent application Ser. No. 07/985,586, now abandoned, filed Dec. 3, 1992, which is a Continuation-In Part of U.S. patent application Ser. No. 07/789,188, now abandoned, filed Nov. 5, 1991 and is also a Continuation-In-Part of U.S. patent application Ser. No. 07/911,533, now abandoned, filed Jul. 10, 1992 and is also a Continuation-In-Part of U.S. patent application Ser. No. 07/787,840, now abandoned, filed Nov. 5, 1991, all of which are incorporated herein by reference. This application also claims priority and is related to PCT/US93/11704, filed Dec. 2, 1993, and is related to PCT/US92/09627, filed Nov. 5, 1992. The teachings of PCT/US92/09267 and PCT/US93/11704 are incorporated herein by reference.

IN: Treco; Douglas A., Heartlein; Michael W., Selden; Richard F.

AB: The invention relates to constructs comprising: a) a targeting sequence; b) a regulatory sequence; c) an exon; and d) an unpaired splice-donor site. The invention further relates to a method of producing protein in vitro or in vivo comprising the homologous recombination of a construct as described above within a cell. The homologously recombinant cell is then maintained under conditions which will permit transcription and translation, resulting in protein expression. The present invention further relates to homologously recombinant cells, including primary, secondary, or immortalized vertebrate cells, methods of making the cells, methods of homologous recombination to produce fusion genes, methods of altering gene expression in the cells, and methods of making a protein in a cell employing the constructs of the invention.

L12: Entry 33 of 45

File: USPT

Jun 24, 1997

DOCUMENT-IDENTIFIER: US 5641670 A
TITLE: Protein production and protein delivery

DEPR:
The identification of the targeting event can be facilitated by the use of one or more selectable marker genes. These markers can be included in the targting construct or be present on different constructs. Selectable markers can be divided into two categories:

positively selectable and
negatively selectable (in other words, markers for either positive selection or negative
selection). In positive selection, cells expressing the positively selectable marker are capable
of surviving treatment with a selective agent (such as neo, xanthine-guanine phosphoribosyl
transferase (gpt), dhfr, adenosine deaminase (ada), puromycin (pac), hygromycin (hyg), CAD which
encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydro-orotase glutamine
synthetase (GS), multidrug resistance 1 (mdrl) and histidine D (hisD), allowing for the selection
of cells in which the targeting construct integrated into the host cell genome. In negative
selection, cells expressing the negatively selectable marker are destroyed in the presence of the
selective agent. The identification of the targeting event can be facilitated by the use of one
or more marker genes exhibiting the property of negative selection, such that the negatively
selectable marker is linked to the exogenous DNA, but configured such that the negatively
selectable marker flanks the targeting sequence, and such that a correct homologous recombination
event with sequences in the host cell genome does not result in the stable integration of the
negatively selectable marker (Mansour, S. L. et al., Nature 336:348-352 (1988)). Markers useful
for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial gpt
gene.

DEPR:
Examples 1f-1h, 2, 4 and 6 illustrate embodiments in which the normal regulatory sequences
upstream of the human EPO gene are altered to allow expression of hEPO in primary or secondary
fibroblast strains which do not express EPO in detectable quantities in their untransfected
state. In one embodiment the product of targeting leaves the normal EPO protein intact, but under
the control of the mouse metallothionein promoter. Examples 1i and 1j demonstrate the use of
similar targeting constructs to activate the endogenous growth hormone gene in primary or
secondary human fibroblasts. In other embodiments described for activating EPO expression in
human fibroblasts, the products of targeting events are chimeric transcription units, in which
the first exon of the human growth hormone gene is positioned upstream of EPO exons 2-5. The
product of transcription (controlled by the mouse metallothionein promoter), splicing, and
translation is a protein in which amino acids 1-4 of the hEPO signal peptide are replaced with
amino acid residues 1-3 of hGH. The chimeric portion of this protein, the signal peptide, is
removed prior to secretion from cells. Example 5 describes targeting constructs and methods for
producing cells which will convert a gene (with introns) into an expressible cDNA copy of that
gene (without introns) and the recovery of such expressible cDNA molecules in microbial (e.g.,
yeast or bacterial) cells. Example 6 describes construction of a targeting vector, designated
pREPO4 for dual selection and selection of cells in which the dhfr gene is amplified. Plasmid
pREPO4 has been used to amplify the human EPO (hEPO) locus in HT1080 cells (an immortalized human
cell line) after activation of the endogenous hEPO gene by homologous recombination. As
described, stepwise selection in methotrexate-containing media resulted in a 70-fold increase in
hEPO production in cells resistant to 0.4 .mu.M methotrexate.

DEPR:
The use of homologous recombination to target a gene of therapeutic interest to a specific
position in a cell's genomic DNA can be expanded upon and made more useful for producing products
for therapeutic purposes (e.g., pharmaceutics, gene therapy) by the insertion of a gene through
which cells containing amplified copies of the gene can be selected for by exposure of the cells
to an appropriate drug selection regimen. For example, pE3neo/hGH (Example 1d) can be modified by
inserting the dhfr, ada, or CAD gene at a position immediately adjacent to the hGH or neo genes
in pE3neo/hGH. Primary, secondary, or immortalized cells are transfected with such a plasmid and
correctly targeted events are identified. These cells are further treated with increasing
concentrations of drugs appropriate for the selection of cells containing amplified genes (for
dhfr, the selective agent is methotrexate, for CAD the selective agent is
N-(phosphonacetyl)-L-aspartate (PALA), and for ada the selective agent is an adenine nucleoside
(e.g., alanosine). In this manner the integration of the gene of therapeutic interest will be
coamplified along with the gene for which amplified copies are selected. Thus, the genetic
engineering of cells to produce genes for therapeutic uses can be readily controlled by
preselecting the site at which the targeting construct integrates and at which the amplified
copies reside in the amplified cells.

DEPR:
Incorporation of a dhfr expression unit into the unique HindIII site of pXEPO-13 (see Example 4)
results in a new targeting vector capable of dual selection and selection of cells in which the
dhfr gene is amplified. The single HindIII site in pXEPO-13 defines the junction of the neo gene
and genomic sequence naturally residing upstream of the human EPO gene. Placement of a dhfr gene
at this site provides a construct with the neo and dhfr genes surrounded by DNA sequence derived
from the natural hEPO locus. Like pXEPO-13, derivatives with the dhfr gene inserted are useful to
target to the hEPO locus by homologous recombination. Such a construct designated pREPO4, is
represented in FIG. 6. The plasmid includes exons 1-4 and part of exon 5 of the human EPO gene,
as well as the HindIII-BamHI fragment lying upstream of the hEPO coding region. pSVe, pTK and
pmMT-I correspond to the promoters from the SV40 early region, the Herpes Simplex Virus (HSV)
thymidine kinase (TK) gene and the mouse metallothionein-I gene. It was produced as follows:
HindIII-digested pXEPO-13 was purified and made blunt with the Klenow fragment of DNA polymerase
I. To obtain a dhfr expression unit, the plasmid construct pF8CIS9080 (Eaton et al., Biochemistry
25:8343-8347 (1986)) was digested with EcoRI and SalI. A 2 Kb fragment containing the dhfr
expression unit was purified from this digest and made blunt with Klenow fragment of DNA
polymerase I. This dhfr-containing fragment was then ligated to the blunted HindIII site of
pXEPO-13. An aliquot of this ligation was transformed into E. coli and plated on ampicillin
selection plates. Following an overnight incubation at 37.degree. C., individual bacterial
colonies were observed, picked and grown. Miniplasmid preparations were made from these cultures
and the resulting DNA was then subjected to restriction enzyme digestion with the enzymes
BglI+HindIII, and SfiI in order to determine the orientation of the inserted dhfr fragments.
Plasmid DNA from one of these preparations was found to contain such a 2 Kb insertion of the dhfr
fragment. The transcription orientation of the dhfr expression unit in this plasmid was found to
be opposite that of the adjacent neo gene. This is the construct designated

pREPO4.

34. Document ID: US 5627073 A

L12: Entry 34 of 45

File: USPT

May 6, 1997

US-PAT-NO: 5627073
DOCUMENT-IDENTIFIER: US 5627073 A
TITLE: Hybridomas producing antibodies to cardiac hypertrophy factor
DATE-ISSUED: May 6, 1997

US-CL-CURRENT: 435/331; 424/139.1, 424/145.1, 435/252.33, 435/332, 435/336, 435/69.6, 435/70.21, 530/387.3, 530/387.9, 530/388.23, 530/391.3

APPL-NO: 8/ 443129
DATE FILED: May 17, 1995

PARENT-CASE:
This is a divisional of co-pending application(s) Ser. No. 08/286,304 filed Aug. 5, 1994 which is a continuation-in-part of 08/233,609 filed Apr. 25, 1994, now abandoned, which application is incorporated herein by reference and to which application priority is claimed under 35 USC .sctn.120.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CHF (also referred to cardiac hypertrophy factor or cardiotrophin-1), isolated DNA encoding CHF, hybridomas and cell lines producing antibodies to CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

L12: Entry 34 of 45

File: USPT

May 6, 1997

DOCUMENT-IDENTIFIER: US 5627073 A
TITLE: Hybridomas producing antibodies to cardiac hypertrophy factor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

35. Document ID: US 5624899 A

L12: Entry 35 of 45

File: USPT

Apr 29, 1997

US-PAT-NO: 5624899
DOCUMENT-IDENTIFIER: US 5624899 A
TITLE: Method for using Htk ligand
DATE-ISSUED: April 29, 1997

US-CL-CURRENT: 514/12; 514/2, 530/350

APPL-NO: 8/ 436044
DATE FILED: May 5, 1995

PARENT-CASE:
CROSS REFERENCES This application is a divisional of co-pending U.S. application Ser. No. 08/277,722 filed 20 Jul. 1994, which application is incorporated herein by reference and to which application priority is claimed under 35 USC 120.

IN: Bennett; Brian D., Matthews; William

AB: A novel hepatoma transmembrane kinase receptor ligand (Htk ligand) which binds to, and activates, the Htk receptor is disclosed. As examples, mouse and human Htk ligands have been identified in a variety of tissues using a soluble Htk-Fc fusion protein. The ligands have been cloned and sequenced. The invention also relates to nucleic acids encoding the ligand, methods for production and use of the ligand, and antibodies directed thereto.

L12: Entry 35 of 45

File: USPT

Apr 29, 1997

DOCUMENT-IDENTIFIER: US 5624899 A
TITLE: Method for using Htk ligand

DEPR:
Briefly, this method involves transforming primary mammalian cells containing endogenous Htk ligand gene (e.g., human cells if the desired Htk ligand is human) with a construct (i.e., vector) comprising an amplifiable gene [such as dihydrofolate reductase (DHFR) or others discussed below] and at least one flanking region of a length of at least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of the Htk ligand gene to provide amplification of the Htk ligand gene. The amplifiable gene must be at a site that does not,interfere with expression of the Htk ligand gene. The transformation is conducted such that the construct becomes homologously integrated into the genome of the

primary cells to define an
amplifiable region.

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

36. Document ID: US 5624806 A

L12: Entry 36 of 45

File: USPT

Apr 29, 1997

US-PAT-NO: 5624806
DOCUMENT-IDENTIFIER: US 5624806 A
TITLE: Antibodies to cardiac hypertrophy factor and uses thereof
DATE-ISSUED: April 29, 1997

US-CL-CURRENT: 435/7.1; 435/331, 435/344.1, 530/387.3, 530/387.9, 530/388.85, 530/391.3

APPL-NO: 8/ 442745
DATE FILED: May 17, 1995

PARENT-CASE:
This is a divisional of application(s) Ser. No. 08/286,304 filed Aug. 5, 1994 which is a
continuation of 08/233,609 filed Apr. 25, 1994 now U.S. Pat. No. 5,534,615, which application is
incorporated herein by reference and to which application priority is claimed under 35 USC
.sctn.120.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CHF, isolated DNA encoding cardiac hypertrophy factor (CHF), and
recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are
shown to influence hypertrophic activity and neurological activity. Accordingly, these
compounds or their antagonists may be used for treatment of heart failure, arrhythmic
disorders, inotropic disorders, and neurological disorders.

L12: Entry 36 of 45

File: USPT

Apr 29, 1997

DOCUMENT-IDENTIFIER: US 5624806 A
TITLE: Antibodies to cardiac hypertrophy factor and uses thereof

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

37. Document ID: US 5605824 A

L12: Entry 37 of 45

File: USPT

Feb 25, 1997

US-PAT-NO: 5605824
DOCUMENT-IDENTIFIER: US 5605824 A
TITLE: Composition for hybridizing nucleic acids using single-stranded nucleic acid binding
protein
DATE-ISSUED: February 25, 1997

US-CL-CURRENT: 435/194; 435/91.2, 530/358

APPL-NO: 8/ 078769
DATE FILED: June 16, 1993

PARENT-CASE:
CROSS REFERENCE TO RELATED APPLICATIONS This application is a continuation of application Ser.
No. 07/603,466, filed Oct. 24, 1990 abandoned which is a continuation-in-part application to
abandoned application Ser. No. 07/425,867, and application Ser. No. 07/425,864, now U.S. Pat. No.
5,449,603, issued Sep. 12, 1995, both filed Oct. 24, 1989 the disclosures of which are thereby
incorporated by reference.

IN: Nielson; Kirk B., Mathur; Eric J.

AB: The present invention relates to an improved composition for hybridizing
polynucleotides with complementary nucleic acid sequences. Specifically, it relates to a
composition for of increasing the specificity of a polynucleotide hybridization reaction in
the presence of single-stranded nucleic acid binding protein.

L12: Entry 37 of 45

File: USPT

Feb 25, 1997

DOCUMENT-IDENTIFIER: US 5605824 A
TITLE: Composition for hybridizing nucleic acids using single-stranded nucleic acid binding
protein

DEPR:
The two primer pairs have sequences that were derived from the human dihydrofolate reductase gene
(DHFR), at exon 1 (pr 1A and pr 1B), or at exon 2 (pr 2A and 2B). However, each primer has 18
nucleotide residues at their 5' ends that are not homologous to the DHFR gene, but rather
correspond in sequence to portions of a universal lambda primer. At the 3' end of each primer
there is a stretch of 33 nucleotides (pr 1A), 27 nucleotides (pr 1B), 27 nucleotides (pr 2A), or
30 nucleotides (pr 2B) that correspond to portions of the human DHFR gene. Thus, when
specifically hybridized to the human genomic DNA DHFR gene, the duplex containing these primers
has a 5' "tail" region comprising 18 nucleotides that is not complementary or hybridized to the
template and is attached to the remaining 3' portion comprising 27 to 33 nucleotides that is
hybridized to the DHFR gene. The above DHFR primers are therefore examples of primers having
substantial complementarity with the target sequence.

38. Document ID: US 5578461 A

L12: Entry 38 of 45

File: USPT

Nov 26, 1996

US-PAT-NO: 5578461
DOCUMENT-IDENTIFIER: US 5578461 A
TITLE: Gene manipulation and expression using genomic elements
DATE-ISSUED: November 26, 1996

US-CL-CURRENT: 435/69.1; 435/244, 435/320.1, 435/464, 536/23.1, 536/24.1

APPL-NO: 8/ 102567
DATE FILED: August 5, 1993

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This is a continuation of application Ser. No. 08/001,898
filed Jan. 7, 1993, now abandoned, which is a continuation in part of application Ser. No.
07/432,069, filed Nov. 6, 1989, now abandoned.

IN: Sherwin; Stephen, Klapholz; Sue, Skoultchi; Arthur

AB: Expression of mammalian target genes is achieved by employing chromosomal target
DNA, either native primary cells or YACs in a yeast host, where the YACs include a fragment
of a mammalian chromosome, the fragment comprising the target gene. Employing homologous
recombination, an amplifiable gene is integrated into the mammalian fragment at a site to
allow for amplification. In the same step, or one or more steps, as desired, the mammalian
gene and/or the transcriptional system may be modified by in vivo mutagenesis. The resulting
construct from homologous recombination may then be transformed into a mammalian expression
host and integrated into the host genome, either randomly or by homologous recombination.
The amplifiable gene may then be amplified by an appropriate agent providing for multiple
copies of the target gene and the expression host grown to provide for high yields of the
desired wild-type or modified protein.

L12: Entry 38 of 45

File: USPT

Nov 26, 1996

DOCUMENT-IDENTIFIER: US 5578461 A
TITLE: Gene manipulation and expression using genomic elements

BSPR:
Mansour et al., Nature, 336:348-352 (1988), describe a general strategy for targeting mutations
to non-selectable genes. Weidle et al., Gene, 66:193-203, (1988), describe amplification of
tissue-type plasminogen activator with a DHFR gene and loss of amplification in the absence of
selective pressure. Murnane and Yezzi, Somatic Cell and Molecular Genetics, 14:273-286, (1988),
describe transformation of a human cell line with an integrated selectable gene marker lacking a
transcriptional promoter, with tandem duplication and amplification of the gene marker. Thomas
and Capecchi, Cell, 503-512, (19871, describe site-directed mutagenesis by gene targeting in
mouse embryo-derived stem cells. Song et al., Proc. Natl. Acad. Sci. USA, 84:6820-6824, (1987),
describe homologous recombination in human cells by a two staged integration. Liskay et al.,
"Homologous Recombination Between Repeated Chromosomal Sequences in Mouse Cells," Cold Spring
Harbor, Symp. Quant. Biol. 49:13-189, (1984), describe integration of two different mutations of
the same gene and homologous recombination between the mutant genes. Rubnitz and Subramani, Mol.
and Cell. Biol. 4:2253-2258, (1984), describe the minimum amount of homology required for
homologous recombination in mammalian cells. Kim and Smithies, Nucl. Acids. Res. 16:8887-8903,
(1988), describe an assay for homologous recombination using the polymerase chain reaction.

39. Document ID: US 5571893 A

L12: Entry 39 of 45

File: USPT

Nov 5, 1996

US-PAT-NO: 5571893
DOCUMENT-IDENTIFIER: US 5571893 A
TITLE: Cardiac hypertrophy factor
DATE-ISSUED: November 5, 1996

US-CL-CURRENT: 530/350; 530/351, 530/399, 930/140

APPL-NO: 8/ 286304
DATE FILED: August 5, 1994

PARENT-CASE:
This application is a continuation of and makes reference to, and claims the

benefits available
under 35 U.S.C. Section 120 of, U.S. Ser. No. 08/233,609, filed 25 Apr. 1994 now U.S. Pat. No.
5,534,615.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of
preparing CHF are disclosed, These CHF molecules are shown to influence hypertrophic
activity and neurological activity. Accordingly, these compounds or their antagonists may be
used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and
neurological disorders.

L12: Entry 39 of 45

File: USPT

Nov 5, 1996

DOCUMENT-IDENTIFIER: US 5571893 A
TITLE: Cardiac hypertrophy factor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

40. Document ID: US 5571675 A

L12: Entry 40 of 45

File: USPT

Nov 5, 1996

US-PAT-NO: 5571675
DOCUMENT-IDENTIFIER: US 5571675 A
TITLE: Detection and amplification of candiotrophin-1(cardiac hypertrophy factor)
DATE-ISSUED: November 5, 1996

US-CL-CURRENT: 435/6; 435/91.2, 435/91.21, 536/24.3, 536/24.31, 536/24.32, 536/24.33

APPL-NO: 8/ 444083
DATE FILED: May 17, 1995

PARENT-CASE:
This is a divisional of co-pending application Ser. No. 08/286,304 filed Aug. 5, 1994 which is a
continuation-in-part of Ser. No. 08/233,609 filed Apr. 25, 1994, which application is
incorporated herein by reference and to which application priority is claimed under 35 USC
.sctn.120.

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennica; Diane, Wood; William

AB: Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of
preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic
activity and neurological activity. Accordingly, these compounds or their antagonists may be
used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and
neurological disorders.

L12: Entry 40 of 45

File: USPT

Nov 5, 1996

DOCUMENT-IDENTIFIER: US 5571675 A
TITLE: Detection and amplification of candiotrophin-1(cardiac hypertrophy factor)

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or
other marker present in the construct. The presence of the marker gene establishes the presence
and integration of the construct into the host genome. No further selection of the primary cells
need be made, since selection will be made in the second host. If desired, the occurrence of the
homologous recombination event can be determined by employing PCR and either sequencing the
resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when
DNA from correct homologous integrants is present and expanding only those cells containing such
fragments. Also if desired, the selected cells may be amplified at this point by stressing the
cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is
DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the
amplification step is not conducted until after the second transformation described below.

41. Document ID: US 5534615 A

L12: Entry 41 of 45

File: USPT

Jul 9, 1996

US-PAT-NO: 5534615
DOCUMENT-IDENTIFIER: US 5534615 A
TITLE: Cardiac hypertrophy factor and uses therefor
DATE-ISSUED: July 9, 1996

US-CL-CURRENT: 530/350; 424/569, 424/570, 530/380

APPL-NO: 8/ 233609
DATE FILED: April 25, 1994

IN: Baker; Joffre, Chien; Kenneth, King; Kathleen, Pennice; Diane, Wood; William

AB: Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

L12: Entry 41 of 45

File: USPT

Jul 9, 1996

DOCUMENT-IDENTIFIER: US 5534615 A
TITLE: Cardiac hypertrophy factor and uses therefor

DEPR:
Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

42. Document ID: US 5482856 A

L12: Entry 42 of 45

File: USPT

Jan 9, 1996

US-PAT-NO: 5482856
DOCUMENT-IDENTIFIER: US 5482856 A
TITLE: Production of chimeric antibodies by homologous recombination
DATE-ISSUED: January 9, 1996

US-CL-CURRENT: 435/320.1; 435/69.1, 435/69.6, 530/387.3, 530/388.8

APPL-NO: 8/ 045945
DATE FILED: April 12, 1993

PARENT-CASE:
The present application is a division of prior application, Ser. No. 07/468,035, filed Jan. 22, 1990, now U.S. Pat. No. 5,202,238, which application is itself a continuation-in-part of prior application, Ser. No. 07/243,873, filed Sep. 14, 1988, now U.S. Pat. No. 5,204,244, which is a continuation-in-part of prior application, Ser. No. 113,800, filed Oct. 27, 1987, now abandoned, each of which is incorporated by reference herein in its entirety.

IN: Fell, Jr.; H. Perry, Folger-Bruce; Kim R., Yarnold; Susan M.

AB: A process for producing chimeric antibodies using novel recombinant DNA vectors and homologous recombination in vivo is described. The recombinant DNA constructs of the invention can be used to transfect antibody producing cells so that targeted homologous recombination occurs in the transfected cells leading to gene modification and the production of chimeric antibody molecules by the transfected cells.

L12: Entry 42 of 45

File: USPT

Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482856 A
TITLE: Production of chimeric antibodies by homologous recombination

DEPR:
Complete "chimerization" of an antibody molecule requires replacement of the light chain constant region as well as the heavy chain. Therefore, vectors were generated to effect chimeric construction of murine kappa light chains by homologous recombination. The first such construct (FIG. 5) was comprised of a 2.7 kb Eco RI fragment containing the exon for human C.kappa., and, as the target sequence, the 2 kb Pst I/Xmn I fragment containing the murine light chain enhancer from the murine light chain intron separating the variable and constant region exons. The vector also contained the bacterial guanosine phosphoryl transferase (gpt) gene derived from the pSV.sub.2 -gpt plasmid (for selection in eukaryotic cells) as well as the ampicillin resistance gene and the bacterial origin of replication from pUC18. A second version of the vector was generated by replacing the gpt gene with a mutated version of the dihydrofolate reductase gene (*DHFR) (FIG. 6). This mutated form of DHFR was originally described by Simonsen and Levinson (1983, Proc. Natl. Acad. Sci. U.S.A. 80:2495-2499) as having a lower affinity for the drug methotrexate than does the wild type gene and which therefore can be used for selection in cells which retain the normal DHFR gene. Stepwise increase of methotrexate concentration has been used to effect the genetic amplification of both DHFR and linked genes with concomitant increase in production of protein encoded by the linked gene. Thus, the mutated DHFR vector offers the advantage of boosting levels of immunoglobulin production via gene amplification.

43. Document ID: US 5449603 A

L12: Entry 43 of 45

File: USPT

Sep 12, 1995

US-PAT-NO: 5449603
DOCUMENT-IDENTIFIER: US 5449603 A
TITLE: Method for hybridizing nucleic acids using single-stranded nucleic acid binding protein
DATE-ISSUED: September 12, 1995

US-CL-CURRENT: 435/6; 536/24.3

APPL-NO: 7/ 425864
DATE FILED: October 24, 1989

IN: Nielson; Kirk B., Mathur; Eric J.

AB: The present invention relates to an improved method for hybridizing
polynucleotides with complementary nucleic acid sequences. Specifically, it relates to a
method of increasing the specificity of a polynucleotide hybridization reaction in the
presence of single-stranded nucleic acid binding protein.

L12: Entry 43 of 45
File: USPT
Sep 12, 1995

DOCUMENT-IDENTIFIER: US 5449603 A
TITLE: Method for hybridizing nucleic acids using single-stranded nucleic acid binding protein

DEPR:
The two primer pairs have sequences that were derived from the human dihydrofolate reductase gene
(DHFR), at exon 1 (pr 1A and pr 1B), or at exon 2 (pr 2A and 2B). However, each primer has 18
nucleotide residues at their 5' ends that are not homologous to the DHFR gene, but rather
correspond in sequence to portions of a universal lambda primer. At the 3' end of each primer
there is a stretch of 33 nucleotides (pr 1A), 27 nucleotides (pr 1B), 27 nucleotides (pr 2A), or
30 nucleotides (pr 2B) that correspond to portions of the human DHFR gene. Thus, when
specifically hybridized to the human genomic DNA DHFR gene, the duplex containing these primers
has a 5' "tail" region comprising 18 nucleotides that is not complementary or hybridized to the
template and is attached to the remaining 3' portion comprising 27 to 33 nucleotides that is
hybridized to the DHFR gene. The above DHFR primers are therefore examples of primers having
substantial complementarity with the target sequence.

44. Document ID: US 5202238 A

L12: Entry 44 of 45
File: USPT
Apr 13, 1993

US-PAT-NO: 5202238
DOCUMENT-IDENTIFIER: US 5202238 A
TITLE: Production of chimeric antibodies by homologous recombination
DATE-ISSUED: April 13, 1993

US-CL-CURRENT: 435/69.6; 435/320.1, 435/463, 435/465, 435/69.7, 435/70.1, 435/70.21, 530/387.3,
530/388.8, 536/23.53

APPL-NO: 7/ 468035
DATE FILED: January 22, 1990

PARENT-CASE:
The present application is a continuation-in-part of copending application Ser. No. 07/242,873,
filed Sep. 14, 1988, and of application Ser. No. 113,800, filed Oct. 27, 1987, now abandoned,
both of which are incorporated by reference herein in its entirety.

IN: Fell, Jr.; H. Perry, Folger-Bruce; Kim R.

AB: A process for producing chimeric antibodies using novel recombinant DNA vectors
and homologous recombination in vivo is described. The recombinant DNA constructs of the
invention can be used to transfect antibody producing cells so that targeted homologous
recombination occurs in the transfected cells leading to gene modification and the
production of chimeric antibody molecules by the transfected cells.

L12: Entry 44 of 45
File: USPT
Apr 13, 1993

DOCUMENT-IDENTIFIER: US 5202238 A
TITLE: Production of chimeric antibodies by homologous recombination

DEPR:
Complete "chimerization" of an antibody molecule requires replacement of the light chain constant
region as well as the heavy chain. Therefore, vectors were generated to effect chimeric
construction of murine kappa light chains by homologous recombination. The first such construct
(FIG. 5) was comprised of a 2.7 kb Eco RI fragment containing the exon for human C.kappa., and,
as the target sequence, the 2 kb Pst I/Xmn I fragment containing the murine light chain enhancer
from the murine light chain intron separating the variable and constant region exons. The vector
also contained the bacterial guanosine phosphoryl transferase (gpt) gene derived from the
pSV.sub.2 -gpt plasmid (for selection in eukaryotic cells) as well as the ampicillin resistance
gene and the bacterial origin of replication from pUC18. A second version of the vector was
generated by replacing the gpt gene with a mutated version of the dihydrofolate reductase gene
(*DHFR) (FIG. 6). This mutated form of DHFR was originally described by Simonsen and Levinson
(1983, Proc. Natl. Acad. Sci. U.S.A. 80:2495-2499) as having a lower affinity for the drug
methotrexate than does the wild type gene and which therefore can be used for selection in cells
which retain the normal DHFR gene. Stepwise increase of methotrexate concentration has been used
to effect the genetic amplification of both DHFR and linked genes with concomitant increase in
production of protein encoded by the linked gene. Thus, the mutated DHFR vector offers the
advantage of boosting levels of immunoglobulin production via gene amplification.

45. Document ID: US 5122463 A

L12: Entry 45 of 45

File: USPT

Jun 16, 1992

US-PAT-NO: 5122463
DOCUMENT-IDENTIFIER: US 5122463 A
TITLE: Methods for trans-destabilization of specific proteins in vivo and DNA molecules useful therefor
DATE-ISSUED: June 16, 1992

US-CL-CURRENT: 435/483; 435/69.7, 536/23.4

APPL-NO: 7/ 525150
DATE FILED: May 17, 1990

IN: Varshavsky; Alexander J., Johnson; Erica S., Gonda; David K., Hochstrasser; Mark

AB: This invention pertains to a method of metabolically destabilizing a protein or peptide of interest in vivo provided that the protein or peptide contains a second determinant of the N-end rule-based degradation signal and provided that another (targeting) protein or peptide can be identified that specifically interacts with the protein or peptide of interest. The methods of the invention comprise contacting the protein or peptide of interest with the targeting protein or peptide which contains a destabilizing amino-terminal amino acid according to the N-end rule of protein degradation but lacking a second determinant of the N-end rule-based degradation signal. Because nearly all proteins specifically interact with other proteins, this is a broadly applicable method for metabolically destabilizing a protein or peptide of interest in vivo.

L12: Entry 45 of 45

File: USPT

Jun 16, 1992

DOCUMENT-IDENTIFIER: US 5122463 A
TITLE: Methods for trans-destabilization of specific proteins in vivo and DNA molecules useful therefor

DEPR:
The discovery of trans targeting and subunit-specific degradation of mature (folded) proteins makes possible a new application of the in vitro findings of Hall and Frieden. Specifically, folding-interfering targeting peptides whose amino acid sequence is identical or substantially homologous to a portion of the amino acid sequence of a protein or peptide of interest, can be used to target the protein of interest, in trans, for either degradation or functional impairment in vivo. In the in vitro approach of Hall and Frieden, the few fragments of DHFR that have actually been used were chosen for reasons of their relative ease of preparation rather than for their expected effect on the DHFR folding. In contrast, our method, in addition to being in vivo-based and protein degradation-oriented, provides for a systematic genetic approach to ascertain directly, for any protein of interest, which of its fragments are the ones that most effectively interfere with the folding, function and/or metabolic stability of the full-length protein.